JOURNAL OF DAIRY SCIENCE

JULY, 1957

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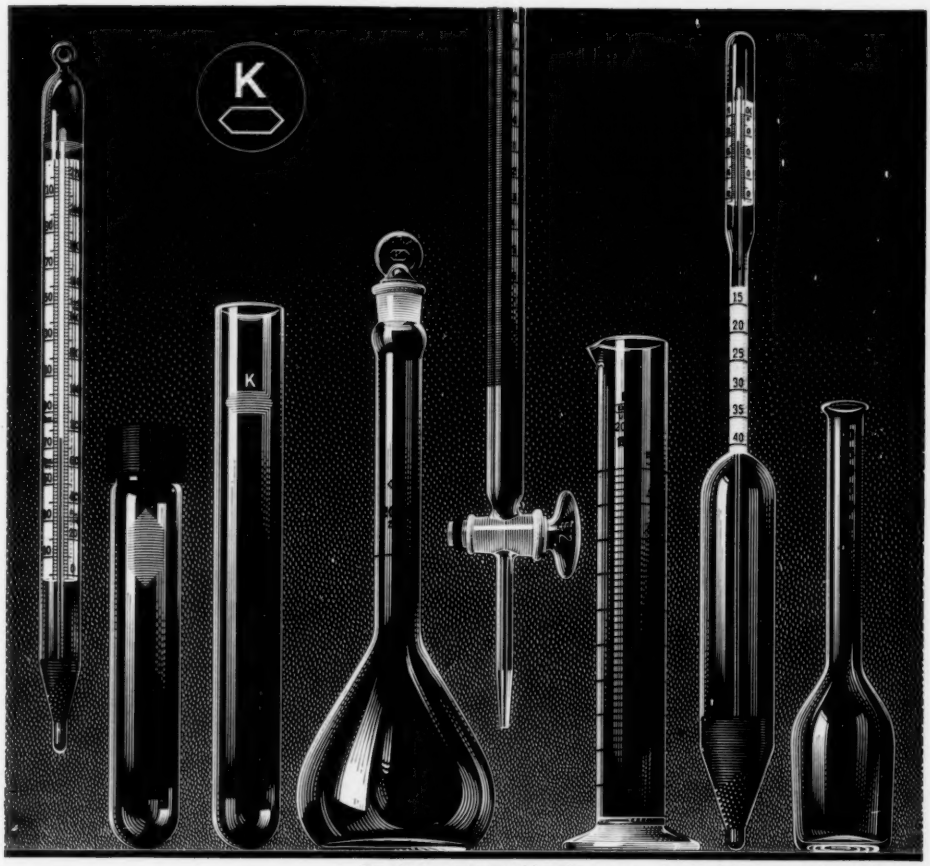

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PEOPLE *and* EVENTS
in the Dairy Science World

Pioneers in the Dairy Industry

On April 12th, ARTHUR H. WHITE was honored by his many friends in the government service and the dairy industry on the occasion of his retirement after over 37 years service.

Born in British Columbia, Mr. White attended Ontario Agricultural College, Guelph, graduating from there in 1917. He enlisted and served overseas in France, Belgium, and Germany with the Canadian Field Artillery. Shortly after demobilization in 1919, he entered the government service. In November of that year he was appointed to take charge of cow testing work under the Dominion Dairy and Cold Storage Branch of the Dept. of Agriculture. In 1922-23 he did postgraduate work at Iowa State College, Ames, from which he received his M.S. degree. In 1925 he was appointed to the Dairy Research Unit, which had recently been set up under the late E. G. HOOD, and carried on in this field of work until his retirement.

ARTHUR H. WHITE

Prior to attending college Mr. White had practical experience with several creameries and milk plants in British Columbia, as well as in cow testing work with the British Columbia Dairy Branch. This practical background served him well in interpreting research findings to the dairy plant operator.

Much of Mr. White's research was on butter and milk powder. In association with Dr. Hood, he made a thorough study of the control and enumeration of yeasts and molds in butter. More recently he led a comprehensive study on the nonwashing of butter, which has promised considerable advantages to the creameryman. Mr. White also served on the Standard Methods Committee of the American Public Health Association and made contributions concerning the procedure for analyzing dried milks.

Mr. and Mrs. White left Ottawa in May to drive to the Pacific Coast for the summer. Both on the way out and back they plan to visit a number of dairy research centers. Mr. White's plans for the future are not definite, but no doubt his vast fund of knowledge and experience will still be useful to the dairy industry.

C. K. JOHNS

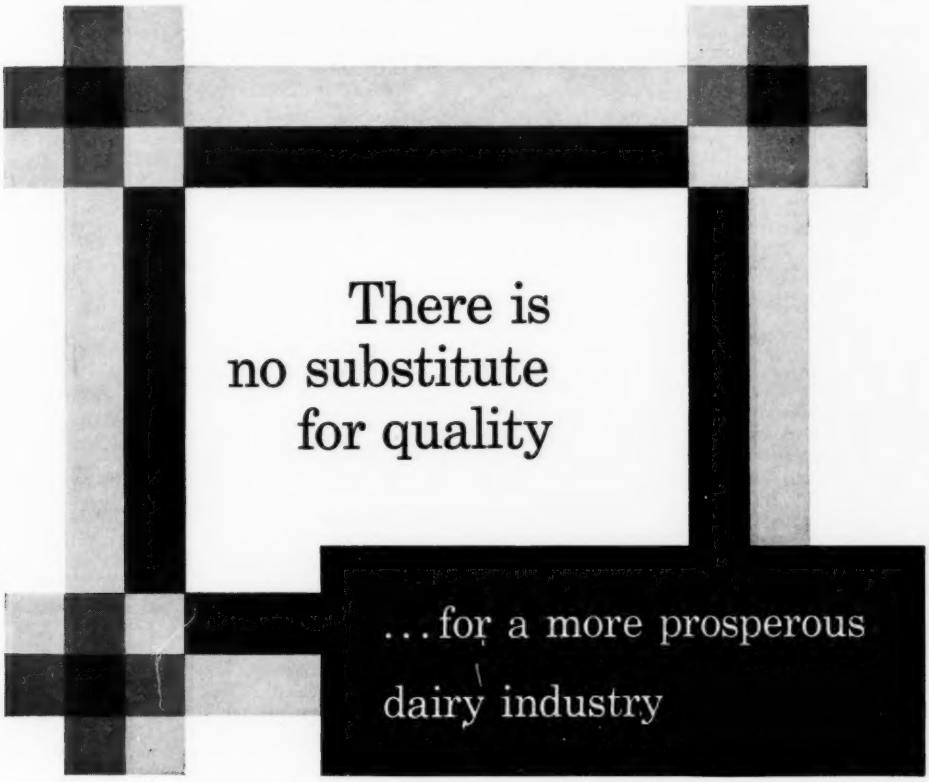
North Carolina State College Improves Facilities

One of the most persistent demands of the dairy industry is for more and better trained personnel. The Dairy Technology Depts. of the universities are doing their best to meet this demand. Most of them have been able to employ competent, aggressive staffs. Many have enlarged and improved their facilities. This increased interest in dairy technology has enabled the industry to recruit more students to the profession.

North Carolina State College, Raleigh, has been able to secure and keep a competent staff in dairy technology. This staff consists of L. W. AURAND, dairy chemist who does teaching and research and was trained at the Univ. of New Hampshire, Durham, and Penn State Univ., University Park; L. F. BLANTON, a North Carolina State College graduate working toward his Ph.D., who teaches and does research in dairy products; R. B. REDFERN, dairy extension specialist, who holds a Ph.D. degree from North Carolina State College; W. M. ROBERTS, teaching, research, and extension in dairy products and management with degrees from Univ. of Tennessee and Univ. of Minnesota; M. L. SPECK, dairy bacteriologist, who teaches and does research with degrees from Univ. of Maryland and Cornell Univ.; and F. G. WARREN, principally teaching in dairy products and counselor of majors in dairy manufacturing, with training at Kansas State College and Penn State Univ. Several graduate students have duties also in the teaching and research programs.

The major emphasis in training is applied

The Dairy Technology Staff and Facilities are Located in Polk Hall at North Carolina State College, Raleigh.



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to the undergraduate and graduate students. The guiding principle in teaching dairy technology is—"Teach a man to live, along with the ability to make a living."

The curriculum is designed to give students an opportunity to take courses in a wide variety of subjects but it also permits each one to choose areas of specialization. All students are required to take courses in English, writing, speaking, mathematics, biology, chemistry, bacteriology, physics, history, economics and psychology. If a student desires, additional courses may be elected in these subjects. In addition to a major in dairy technology, the students can choose to specialize in economics and business administration, the sciences such as bacteriology and chemistry which can lead to graduate programs and research jobs, or dairy production. The flexibility of the curriculum permits the student and his counselor to adapt the program to the student and not make the student fit the program. Graduate students choose their major in dairy bacteriology, dairy chemistry, dairy products or dairy plant management.

North Carolina State College has kept pace in providing facilities for teaching and research. An addition to the dairy plant for the fluid milk and ice cream operations was completed and occupied within recent months. The rooms that were vacated will be renovated and used for teaching, pilot plant operations and storage.

In planning the building, an attempt was made to include the latest technological advances in design, construction and layout for maximum ease of operation and sanitation. The equipment was located to provide centralization of steam, water and electric lines and also to allow plenty of room for working and instructional areas. Overflow and drain lines are piped to drains so that there will be a minimum of water on the floor. This should make working conditions safer and more pleasant. Cleaning in-place lines and mechanical cleaning have been incorporated for most of the equipment and floors.

The equipment is of the latest design. It was secured from different manufacturers so that students and short course trainees would have access to a wide variety. It is of such capacity and design that students can get training that will permit them to adapt quickly to commercial dairy operations.

Since all of the milk is received in tanks from producers, the plant does not have a receiving room.

The principal uses made of the dairy plant are:

1. Teaching—(a) Laboratory courses for undergraduate and graduate students; (b) Short courses and conferences such as Market Milk, Ice Cream, Milk Sanitarians, Laboratory Technicians, and Cottage Cheese.
2. Research and pilot plant operations.
3. Tours for school children to acquaint them with processing of milk and dairy products.
4. College students get experience while earning a part of their way through school.

News from Veterans of the A. D. S. A.

In response to invitations to sit on the platform during the awards night program at Oklahoma A & M College on June 29th, H. F. JUDKINS received letters from the following members:

E. S. GUTHRIE—"Thank you for the invitation. I am sorry that our golden wedding anniversary will interfere with my attending this year. That great event for Mrs. Guthrie and me occurs on June 20th."

C. C. HAYDON—"Although I appreciate the thoughtfulness of the invitation to be with you, I regret that my health will not permit it."

C. W. HOLDAWAY—"You can be assured that I appreciate very much your kind invitation. It is with great regret that I must decline. I am unable to travel any distance due to advice of my doctor. This has been true for the last two years. I appreciate more than I can express the honor of being a life member of the A.S.D.A."

O. E. REED—"Thanks for the invitation. Sorry it will not be possible for me to accept. I would like very much to be at the meeting and had planned to be there, but other duties over which I am not master prevent me from carrying out good intentions. I am feeling fine."

H. A. HARDING (Mrs. Harding writes)—"Thank you very much for inviting Dr. Harding. It will be impossible for him to attend as he does not take long trips anymore. He still reads the Journal. He sends his best regards to all who remember him and regrets that he cannot be with you on this memorable occasion."

W. A. WENTWORTH—"I am very sorry that I cannot attend the annual meeting this year. Mrs. Wentworth and I recently returned from a trip to Salt Lake City, where we attended a meeting of the Sons of the American Revolution. We saw Bryce and Zion Canyons and the north rim of the Grand Canyon. Had a most enjoyable trip. Greetings to all."

Poulton Named Head of Animal Industry at Maine

BRUCE R. POULTON, associate professor of dairy husbandry, has been appointed head of the Dept. of Animal Industry at the Univ. of Maine, Orono.

Dr. Poulton replaces HOWARD C. DICKEY as department head who has asked to be relieved of administrative duties in order to devote more time to research and teaching.

Dr. Poulton completed both his undergraduate and graduate work at Rutgers Univ., New Brunswick, N. J. Joining the staff on July 1, 1956, Prof. Poulton has been teaching in the College of Agriculture and doing research in the Agricultural Experiment Station in animal nutrition.

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Finalists in Connecticut's Dairy Club Queen Contest. Front, *left to right*—P. BROWN, J. HOUSE, P. NESTER; rear, *left to right*—M. LEONARD, J. SEMENTINI (winner).

Connecticut Dairy Club News

The annual Dairy Club Breakfast was held this Spring at the Univ. of Connecticut, Storrs. The club honored two outstanding industry men—G. SIMPSON in production, and G. E. SARTAIN in manufacturing. Their pictures were hung in the Animal Industries building and the Dairy Club Yearbook was dedicated to them.

The breakfast was attended by over 250 members of the industry, club members, and the university staff. This year, in cooperation with the state milk industry and the Dairy Club, a Dairy Queen—Miss J. SEMENTINI—was chosen from among university co-eds at the breakfast by a majority vote of those present. She appeared at functions throughout the state in conjunction with June Dairy Month, as well as on television and radio.

During the afternoon, the Dairy Club sponsored the Annual Fitting and Showing Contest in which any student in the university may show an animal which has been fitted and trained during the winter and early spring. The Dairy Club Queen distributed ribbons and prizes to the winners. Between classes the Faculty-Student milking contest was held and won by the students, breaking a consecutive string of wins by the faculty. The can-rolling contest and the milk pail relay were featured contests. Grand champion of the show was L. TREPP with an Ayrshire heifer, and reserve champion, W. RAINVILLE, with a Holstein.

Recent topics presented at the bi-weekly club meetings have included: "The Operation of a Modern Milk Plant" by R. RADWAY, owner of Radway's Dairy; "Advertising and Selling of Dairy Products" by C. SHELDON, owner of Toll Gate Dairy Farms; "Extension on the County Level in Dairying" by J. ELLIOTTE, County Agricultural Extension Agent; "The Operation of a Loose Housing Unit" by K. ANDRUS, dairy farmer; "The Operation of a Producer-Dealer Dairy" by R. FALK, dairyman and retailer, and pioneer in milk vending machines; and "Farming on the Isle of Jersey" by E. L. MARSH, Jersey breeder and twice a visitor to the home of the Jerseys.

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New Appointment in National Dairy Council

W. E. EPPLE, assistant director of public relations for the National Dairy Council, has been appointed Director of Information. The new Dept. of Information includes all communication activities formerly conducted by the Dept. of Public Relations, which was eliminated.

LLOYD H. GEIL, N.D.C. Director of Public Relations since 1948, resigned June 1 to become General Manager of the Poultry and Egg National Board, St. Charles, Ill.

EDWARD A. WELLS, Chicago, a member of the Jewel Tea Co. here, has been awarded the LaChoy Food Products $1,500 scholarship for one year's study in the special Food Distribution Curriculum at Michigan State Univ., E. Lansing, Mich. Mr. Wells was chosen from more than 200 applicants.

Purpose of the scholarship is to aid in the development of outstanding young people to meet the rapidly expanding demand for executives in the food industry, explained Edward M. Muldoon, general manager of LaChoy, leading manufacturer of canned American-Chinese foods.

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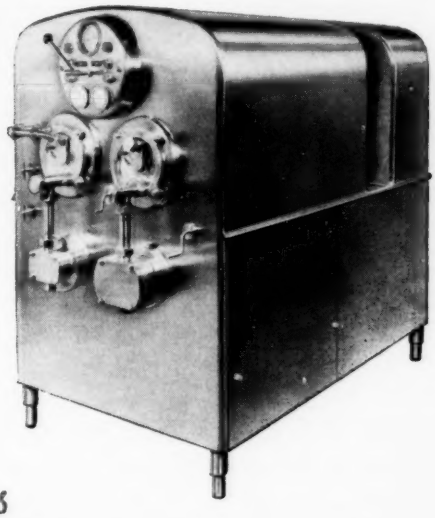

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Marsden Appointed Secretary of 15th International Dairy Congress

A. W. MARSDEN, M.Sc., D.I.C., A.R.C.S., F.R.I.C., director of the Commonwealth Bureau of Dairy Science and Technology at Shinfield, Reading, England, has been appointed organizing secretary of the 15th International Dairy Congress to be staged in London, June 29-July 3, 1959.

The Congress is sponsored by the United Kingdom Dairy Association, which is the British National Committee for affairs of the International Dairy Federation, and has secured the gracious patronage of Her Majesty the Queen.

Mr. Marsden was appointed Director of the Bureau in 1950 after being on the staff of the Imperial College, London, and of Seale-Hayne Agricultural College, Devon. During the war he served as a naval officer. While at the Bureau, Mr. Marsden has been in close touch with the scientific, technical and administrative sides of the dairy industry both at home and abroad. He has visited dairying centers in many overseas countries in recent years, including Canada, U.S.A., Australia, New Zealand, India, Pakistan and several European countries and Colonial Territories. In 1951 he was a U.K. delegate on O.E.E.C. Technical Assistance Mission to the U.S.A.

For some years Mr. Marsden has taken an active part in several learned societies in Britain, and has published a number of papers surveying agricultural production and dairying developments. He has been a member of Council and of a number of Committees of the Society of Chemical Industry, including being Chairman of the Agricultural Group, and Chairman of the Journals and "Chemistry & Industry" Sub-Committee.

Mr. Marsden took up his new appointment on 1st July 1957 and Mr. E. J. MANN, N.D.D., at present Assistant Director, becomes Acting Director of the Bureau.

News from Beatrice Foods

WILLIAM F. NADISCH has been elected president and general manager of Meadow Gold Ice Cream Co., Baltimore, Md., a division of Beatrice Foods Co. He succeeds M. Jacob Abrams, who passed away May 20, 1957.

JAY G. NEUBAUER, Chicago, and LOUIS KOMMINSK, New Bremen, Ohio, have been elected vice presidents of the Beatrice Food Company.

Neubauer, a veteran of 33 years service with Beatrice Foods, will supervise five districts as Central Regional Vice President. They are the Northern District, the Southern District, the Illinois District, the Kansas-Nebraska-Oklahoma District and the Iowa and Missouri District. He is a graduate of the Univ. of Illinois, Urbana.

Komminsk is manager of the Indiana-Southern Ohio District. He began working summers at Beatrice Foods' New Bremen plant in 1929

while still attending school. He received his Bachelor of Science degree in Business Administration in 1937 and a Bachelor of Science degree in Dairy Technology in 1940 from Ohio State Univ.

Borden Scholarship Awards

A total of 1,344 college and university students have received Borden Company Foundation scholarship awards and prizes over the past 13 years.

There are 176 awards made annually and they are administered by 113 different colleges and universities in 38 states. The purposes of the program are to furnish incentive and recognition for outstanding academic attainment, and to emphasize its importance.

Most of the awards go to senior students who stand highest in their class for the first three years of college work. These students receive $300 each.

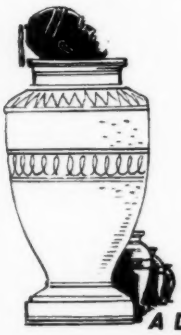
Eligible fields for these awards are agriculture, home economics, journalism, pharmacy, and veterinary medicine. There are also $200 awards at 31 schools for the freshman student who has attained the highest average marks in his class for the first year of college work.

The freshman awards, begun on an experimental basis in 1956, seek to recognize at the very beginning of the college career the importance of good scholarship. Additionally, there is a group of eight graduate and special awards in various fields. In all instances, the colleges select the award students and administer the grants.

L. C. Mitchell Wins First AOAC-Wiley Award

LLOYD C. MITCHELL, research chemist of the Food and Drug Administration, USDA, has been selected to receive the annual Harvey W. Wiley Award of the Association of Official Agricultural Chemists. Mr. Mitchell is the first winner of the award, which was established last year to honor the father of the original Pure Food and Drug Law. The award consists of $500 in cash and goes to the scientist who makes an outstanding contribution to the development of methods for the analysis of foods, drugs, cosmetics, feeds, fertilizers, pesticides, and soils, or for use in general analytical chemistry.

The announcement was made by M. P. ETHEREDGE, Dean of Science at Mississippi State College and President of the Association of Official Agricultural Chemists. He said in notifying Mr. Mitchell, "During more than 40 years of public service, you have made substantial contributions to methodology and have developed and carried through the phase of collaborative studies what have become official methods of analysis."



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Spring Events at Minnesota

During the spring quarter at the Univ. of Minnesota, St. Paul, the Dairy Science Club members took part in many special events. The Milk Hour, which was held in May, drew the largest crowd ever attained at the St. Paul Student Union for this event. "Princess Kay of the Milky Way," BARBARA JEAN BOSSUS, Minnesota's Dairy Princess, was present to serve milk. The Milk Hour will continue to be a quarterly event.

Minnesota Royal Dairy Showmanship was judged this year by Mr. BLAKESLY of the American Breeders Service. The Grand Champion Showman in Dairy was JERROLD JOHNSON, sophomore in agriculture.

Two of the Dairy Science Club members, DALE BLANK, former president of the club and a senior in Dairy Husbandry, and DONALD OTTERNESS, sophomore in dairy husbandry, took part in June Dairy Month events. They served as attendants to the June Dairy Month mascot, Ivanhoe Berry, an Ayrshire cow, during her appearances in Minneapolis.

Dale Blank accompanied a Holstein calf, which was presented to President DWIGHT D. EISENHOWER, when it was flown to New York and Washington, D. C. All of these events were sponsored by the Minnesota Dairy Industry Committee.

The All-Dairy Picnic was held May 23 at Como Park with over 100 in attendance. This was a fitting climax to the school year and was enjoyed by all.

Miss BARBARA JEAN BOSSUS, Minnesota's Princess Kay of the Milky Way, serving milk at the Milk Hour.

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Colorado State Univ. Begins Dairy Research Program

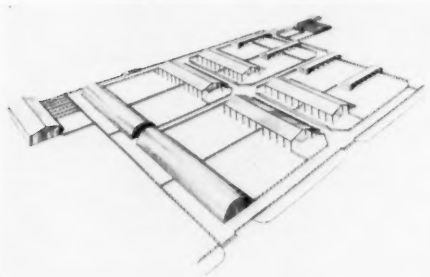

Illustration of Colorado State University's new farm and dairy facilities at Ft. Collins, Colo.

The building expansion program of the Colorado State Univ. dairy and farm facilities, under construction near Ft. Collins, Colo., will enable the university's scientists to do more extensive and fruitful research in the dairy field than has been possible in the past, according to E. K. McKellar, associate dairy husbandman.

The dairy research expansion is the first part of a long-range ten million dollar program for academic and research structures for the university. The new five-acre development is owned by the university.

The new facilities are expected to be particularly productive in research on feeding dairy stock. They will include, besides the milking barn-garage, six resting barns, four of which will be 24 ft. by 80 ft., and the other two 24 ft. by 160 ft.; four hay storage barns, each 32 ft. by 120 ft.; one experimental barn, 24 ft. by 120 ft.; and one calf barn, 32 ft. by 100 ft. Seen off a short distance from the dairy buildings will be the Farm Center which will have two wings; one, a shop, 40 ft. by 100 ft.; the other, for machinery storage, 32 ft. by 120 ft.

The space allotted for cows in the new facilities will maintain the highest degree of sanitation and cattle health. A portion of five lots and sheds will allow the separation of research animals for controlled experiments. The buildings for the most part have steel frames.

Scholarship Fund Given to Univ. of Tennessee

The Tennessee Dairy Products Association has presented $900 to the Univ. of Tennessee, Knoxville, to be awarded as three $300 scholarships to juniors majoring in dairy manufacturing. The check was presented to President C. E. Brehm, Univ. of Tennessee, by Russell Brothers, president of the Dairy Products Association, at a banquet of the U. T. Dairy Club in May.

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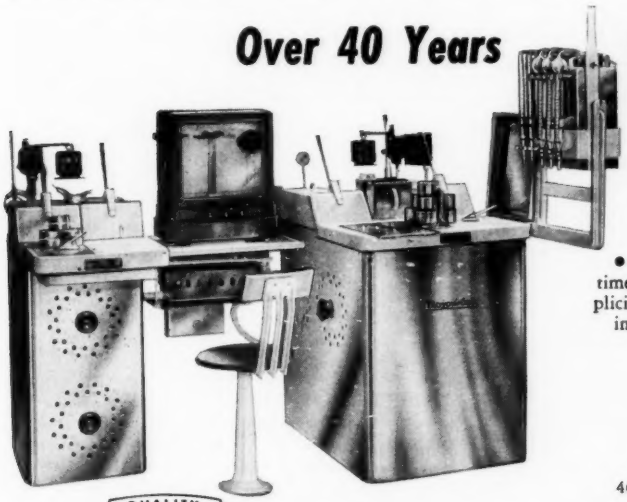

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Oklahoma State News

Ten Oklahoma State Univ. students received the first dairy manufacturing scholarships ever offered by the university in this field. They were made available by a $2,400 grant to OSU by the Oklahoma Dairy Products Institute.

Realizing a need for more trained men in dairy manufacturing, the Oklahoma Dairy Technology Society last year co-sponsored, with OSU, a brochure which listed opportunities and requirements for careers in dairy manufacturing. As a result of this publicity, the Dairy Dept. had more than 30 applicants for scholarships for 1957-58. Six freshmen and two sophomores received $175 each, and two juniors and two seniors received $250 each.

Freshmen selected for scholarships were R. MORGAN, T. HERTZ, W. MARLEY, K. BOSTON, W. FOWLER, AND L. DRESSLER.

P. MCCOMAS, R. JAMESON, S. ALLEN, and J. ROBISON received scholarships to continue their studies in dairy manufacturing.

Recipients of dairy manufacturing scholarships at Oklahoma State Univ. are congratulated by H. C. OLSON, dairy manufacturing professor. PAT MCCOMAS receives his $250 scholarship and SANFORD ALLEN waits as C. L. NORTON, head of the Dairy Dept., signs the awards.

Babcock Dairy Science Club Activities

"Alice in Dairyland," Wisconsin's number one promoter of dairy products, Miss DORIS OLSON, was the guest speaker at the final meeting of the Babcock Dairy Science Club of the Univ. of Wisconsin, Madison. She stated that hospitality in the South was exceeded by expectations as she made personal appearances and gave samples of Wisconsin cheese. In Texas, she was flown from town to town to speak with people, answer questions about Wisconsin dairy products, and talk at displays put up by the Wisconsin Dept. of Agriculture.

The annual Spring banquet, held jointly with the Food Technology Club, was a great success. The graduating seniors were honored and some faculty members gave a few parting words. More funds were obtained by the sale of refreshments at Prof. Gordon Day, which is an annual children's spring music festival.

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HERITABILITY, GENETIC AND PHENOTYPIC CORRELATIONS OF CERTAIN CONSTITUENTS OF COW'S MILK[1]

K. R. JOHNSON

Department of Dairy Husbandry, University of Idaho, Moscow

The average production for 152 Holsteins was 14,485 lb. of milk, containing 3.34% fat and 8.58% SNF, and for 140 Jerseys it was 6,487 lb. of milk, containing 5.45% fat and 9.45% SNF. The heritability of each characteristic and the phenotypic and genetic correlations between different characteristics were derived on an intra-sire basis. The Holsteins had the following heritabilities: milk, 0.30; fat, 0.30; % fat, 0.33; SNF, 0.35; % SNF, 0.34; TS, 0.34, and % TS, 0.38. Heritabilities for the Jerseys for these seven characteristics were: 0.26, 0.33, 0.33, 0.34, 0.36, 0.37, and 0.36, respectively. The high genetic correlation between milk production and its constituents, and between constituents, indicates that single genes affect all of them. *Editor.*

The manufacturers of dairy products realize that a more equitable pricing system must be devised which takes into consideration the nutritional value of both the fat and solids-not-fat portions of milk. It has been proposed that a portion of the total price paid for milk be on the basis of the solids-not-fat content. If a pricing system of this type is adopted, dairy-cattle breeders will undoubtedly strive to increase the solids-not-fat portion of milk. To accomplish this, it will be essential for them to know the heritability of the solids-not-fat content of milk, as well as the genetic and phenotypic correlations of total milk and butterfat production to the solids-not-fat content.

Constructive breeding should have for one of its goals the production of an animal which manufactures products for human consumption more economically, and with a longer productive life. This is a difficult problem with dairy cows, because the characters responsible for a large flow of milk, including the constituents of milk, are not completely understood.

The problem becomes even more difficult when trying to improve two or more characteristics simultaneously. The progress made when selecting for two or more characteristics depends primarily on the heritabilities of these characteristics, the genetic correlation between them in the same individual, and the actual intensity of selection. This study was an attempt to estimate the heritability, genetic and phenotypic correlations of milk production and of some of the various constituents of milk.

Genetic correlations in this study mean the correlations between the sets of genes which affect two characteristics in the same animal. Genetic correlations

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[1] Published with the approval of the Director of the Idaho Agricultural Experiment Station as Research Paper No. 413.

should not be confused with phenotypic correlations, since phenotypic correlations between traits are a result of a combination of genetic and environmental correlations. The method used to estimate the genetic correlations involves only the average or additive effects of these sets of genes, plus some of their epistatic effects; because only these contribute to the likeness between daughter and dam, which was the basis of these estimations.

Two different kinds of genetic correlations are: *(a)* those produced by the pleiotropic action of genes, and *(b)* those due to the gradual way in which genetic equilibrium is approached by genes associated because of the manner in which they were introduced into a population. Correlations due to linkage are a case of the latter kind. Only in a population where either the coupling or the repulsion phase of the double heterozygote is far more abundant than the other, can linkage be an important cause of genetic correlations. A condition of this type would persist for only a few generations after a cross because, in a freely interbreeding population, the coupling and the repulsion phases of the double heterozygote tend rapidly to become equally frequent. The different groups of genes can be made rare or abundant in the different subgroups by selection intensities or directions of selection in the noninterbreeding subgroups of a population.

As pointed out by Touchberry *(11)*, when the whole population is considered as a unit, these group differences in gene frequency would contribute to genetic correlations, neglecting differences between subgroups; but would not cause genetic correlations within subgroups, each considered as a unit by itself. Lindstrom *(6, 7)* and Green *(2)* have published work showing genetic correlations between characteristics, involving a qualitative and a quantitative characteristic. In these cases they attributed the genetic correlations to the linkage of qualitative genes with the quantitative genes, but the results could have been due to the pleiotropic effects of genes.

The term "pleiotropy" is used to cover the diversity of action of a single gene. It is now known that a given unit can have effects on different parts of the organism which differ to some degree. Effects of this kind are called "pleiotropic." Since the number of observable characteristics in an organism are infinite, while the number of genes is limited, many of the genes must affect not merely one characteristic but several at a time. Gruneberg *(3)* proposes three ways in which a gene may produce pleiotropic effects: (*a*) A gene may affect two characteristics directly but in independent ways; (*b*) a gene may affect two characteristics in essentially the same way; its primary product may affect them alike; (*c*) a gene may affect one characteristic directly and this characteristic will affect another characteristic. Gruneberg calls the first method of action "genuine pleiotropy" and the other two "spurious pleiotropy."

Hazel *(4)*, by correlating the phenotypic expressions of one character in one animal with the phenotypic expressions of another character in a closely related animal, obtained estimates of genetic correlations of 0.51 between score and weight, and correlations of zero between weight and productivity and score and productivity in swine.

Tyler and Hyatt (*12*), using Hazel's method, found the genetic correlation between milk and percentage of fat to be –0.20; between milk and fat production, 0.85; and between butterfat and percentage of butterfat, 0.26. The corresponding phenotypic correlations were –0.14, 0.93, and 0.23.

Moore and Morrow (*9*) indicate from their study that the factors, milk production, percentage of butterfat, and percentage of solids-not-fat may be inherited separately.

Black and Voris (*1*) found in Holstein cows phenotypic correlations of 0.90 between butterfat and total solids and of 0.68 between butterfat and solids-not-fat. They reported that as percentage of butterfat increases, percentage of total solids and solids-not-fat increases; as percentage of total solids increases, percentage of solids-not-fat increases. Overman and Sanman (*10*) obtained a coefficient of correlation of 0.96 between the total solids and butterfat percentages. White and Judkins (*13*), in a study of 49 Ayrshire, Guernsey, Holstein, and Jersey cows with a total of 127 lactations, found an average percentage of butterfat and solids-not-fat of 4.10 and 8.82 for Ayrshires, 4.49 and 8.92 for Guernseys, 3.41 and 8.53 for Holsteins, and 5.32 and 9.07 for Jerseys. With individuals, the solids-not-fat are more constant than the fat.

EXPERIMENTAL PROCEDURE

Milk samples used in this study were collected from the University of Idaho herd during the periods of 1935 through 1942 and 1953 through 1955. (The study was discontinued during World War II and renewed in 1953.) Two-day composite samples of milk were collected from each cow each month for the first 305 days of that lactation. A total of 1,620 samples was collected from 76 daughter-dam pairs of the Holstein herd and 1,400 samples from 70 daughter-dam pairs of the Jersey herd. The samples were made up of aliquot proportions from each milking of the individual cow during the two-day period, and contained at least 250 cc. of milk. The samples were analyzed for percentage of butterfat by the Babcock method and percentage of total solids by the Mojonnier method, the difference between the two tests being the solids-not-fat fraction. The total production records were adjusted for age at calving, using correction factors derived by Kendrick (*5*). The 76 Holstein daughters were from 8 different sires and 76 different dams, and the 70 Jersey daughters were from 8 different sires and 70 different dams.

ANALYSIS OF DATA

Intra-sire phenotypic correlations between the various constituents of milk and milk production were computed, to see which of these constituents varied with milk production. The phenotypic correlations between the various constituents of milk were next computed, to determine which of these constituents were phenotypically related. The intra-sire correlations between the seven variables in the dams and the seven variables in the daughters were next computed. Intra-sire correlations were used so as to avoid any environmental trends that may have occurred over the periods studied. From these data, heritability estimates and genetic correlations were determined.

One of the difficult problems in analyzing these data was to estimate the genetic correlations between the various constituents of milk studied. The phenotypic correlations between different constituents of the milk of the same animal cannot be used, since they obviously contain an enviromental component as well as the genetic component. Hazel (*4*) has suggested the use of close relatives to avoid the effects of the common environment on characteristics of the same animal. Touchberry (*11*) used a modification of Hazel's formula in reporting genetic correlations between the various characteristics of type in dairy cattle. The genetic correlations reported in this study were determined by means of Touchberry's formula.

The correlations between the seven variables in the dams and the seven variables in the daughters, required when this formula is used, were computed on an intra-sire basis as described by Lush (*8*). Using the appropriate correlations from Tables 1 and 2 in this formula, estimates of the genetic correlations were made and these estimates are given (Tables 3 and 4). As an example of the calculations, the genetic correlation between milk and butterfat production for the Holstein herd is derived by substituting the appropriate figures from Table 1 in the formula, thus:

$$\sqrt{\frac{(0.226)\quad(0.040)}{(0.153)\quad(0.153)}} = 0.620$$

RESULTS AND DISCUSSION

The intra-sire correlations of dam and daughter for the seven characteristics studied in the two breeds are shown (Tables 1 and 2). These data were deter-

TABLE 1

The intra-sire correlations between the seven characteristics measured on different but related (daughter and dam) animals in the Holstein herd

Dam		Daughter						
Characteristic	Symbol	A	B	C	D	E	F	G
Milk	A	0.153	0.001	0.226	0.062	0.170	0.029	0.162
% Fat	B	−0.187	0.166	0.055	0.142	−0.081	0.161	−0.031
Fat	C	0.040	0.222	0.153	0.173	0.138	0.170	0.161
% Solids-not-fat	D	−0.019	0.142	0.147	0.171	0.151	0.170	0.168
Solids-not-fat	E	0.159	0.044	0.190	0.144	0.177	0.112	0.175
% Total solids	F	−0.135	0.100	0.158	0.190	0.099	0.191	0.098
Total solids	G	0.160	0.115	0.162	0.171	0.199	0.152	0.174

Correlations $\geqq 0.223$ are significant at the 0.05 level of probability.
Correlations $\geqq 0.290$ are significant at the 0.01 level of probability.

Characteristic	Intra-sire correlation	Heritability	Intra-sire regression	Heritability
A	0.15	0.30	0.13	0.26
B	0.16	0.32	0.18	0.36
C	0.15	0.30	0.14	0.28
D	0.17	0.34	0.18	0.36
E	0.17	0.34	0.18	0.36
F	0.19	0.38	0.18	0.37
G	0.17	0.34	0.19	0.38

TABLE 2

The intra-sire correlations between the seven characteristics measured on different but related (daughter and dam) animals in the Jersey herd

Dam		Daughter						
Characteristic	Symbol	A	B	C	D	E	F	G
Milk	A	0.133	0.140	0.138	0.160	0.125	0.203	0.180
% Fat	B	−0.206	0.167	0.067	−0.096	−0.007	0.031	0.013
Fat	C	0.138	0.147	0.169	0.216	0.170	0.237	0.170
% Solids-not-fat	D	−0.041	0.217	−0.027	0.181	−0.002	0.158	−0.002
Solids-not-fat	E	0.121	0.118	0.171	0.216	0.174	0.206	0.183
% Total solids	F	0.003	0.142	0.004	0.200	−0.009	0.181	0.007
Total solids	G	0.101	0.184	0.176	0.258	0.160	0.221	0.189

Correlations $\geqq 0.250$ are significant at the 0.05 level of probability.
Correlations $\geqq 0.325$ are significant at the 0.01 level of probability.

Characteristic	Intra-sire correlations	Heritability	Intra-sire regression	Heritability
A	0.13	0.26	0.11	0.23
B	0.16	0.32	0.14	0.28
C	0.16	0.32	0.15	0.30
D	0.18	0.36	0.17	0.34
E	0.17	0.34	0.17	0.34
F	0.18	0.36	0.17	0.34
G	0.18	0.36	0.17	0.34

mined from milk samples taken on a total of 76 daughter-dam pairs of the Holstein breed and 70 daughter-dam pairs of the Jersey breed.

Estimates of heritability for the seven characteristics can be made by doubling the seven correlations on the diagonal from the upper left- to the lower right-hand corner of Tables 1 and 2. The estimates of heritability for the seven characteristics studied for the two breeds are also reported (Tables 1 and 2). A search of the literature did not reveal any previously reported estimates of heritability for solids-not-fat or total solids, or for the percentages of these two constituents of milk. The estimates of heritability for milk, fat, and percentage of fat fall in the ranges reported by other workers.

Estimates of the genetic correlations of the seven characteristics studied for the two breeds were made, using the appropriate correlations from Tables 1 and 2 in Touchberry's (*11*) formula, and are reported in Tables 3 and 4. He makes the following statement concerning use of this formula: "When one figure in the numerator is negative and the other positive, the arithmetic mean of the two figures in the numerator is used in place of their geometric mean to avoid the difficulty of the square root of a negative number. When both figures in the numerator are negative, the geometric mean is used just as when all the figures are positive except, of course, the genetic correlations are given a negative sign."

All of the genetic correlations involving milk and the three constituents of milk studied were positive and large for both the Holstein and Jersey herds. Since butterfat, solids-not-fat, and total solids are constituents of milk, large, positive genetic correlations would be expected and may be termed largely automatic. The genetic correlations between milk and percentage of butterfat were negative for the Jersey herd and negative and large for the Holstein herd. These

TABLE 3

The phenotypic[a] and the genetic correlations between the seven characteristics for the Holstein herd

Characteristics		B	C	D	E	F	G
Milk	P	−0.063	0.751	−0.081	0.958	−0.139	0.935
A	G	−0.584	0.620	0.135	0.993	−0.309	0.987
% Fat	P		0.537	0.365	−0.024	0.839	0.159
B	G		0.691	0.720	−0.111	0.848	0.247
Fat	P			0.184	0.789	0.421	0.820
C	G			0.981	0.987	0.959	0.993
% Solids-not-fat	P				0.200	0.830	0.204
D	G				0.844	0.994	0.982
Solids-not-fat	P					0.101	0.978
E	G					0.173	0.994
% Total solids	P						0.212
F	G						0.670
Total solids	P						
G	G						

[a] Phenotypic correlations ≥ 0.23 and 0.31 are significant at the 0.05 and 0.01 levels of probability, respectively.

negative correlations would be expected, since it is a known fact that with higher milk production there is a lower percentage of the fat both within and between breeds. Genetic correlations between milk and percentage of solids-not-fat were positive for both herds and large for the Jersey herd. Between milk and percentage of total solids the correlations were negative and large for the Holstein herd and positive, but small, for the Jersey herd. With the relatively small number of daughter-dam pairs, sampling errors could mask these correlations and

TABLE 4

The phenotypic[a] and the genetic correlations between the seven characteristics for the Jersey herd

Characteristics		B	C	D	E	F	G
Milk	P	−0.289	0.916	−0.106	0.981	−0.169	0.973
A	G	−0.288	0.920	0.387	0.809	0.154	0.849
% Fat	P		0.229	0.375	−0.101	0.864	0.021
B	G		0.589	0.350	0.329	0.379	0.273
Fat	P			0.056	0.925	0.183	0.973
C	G			0.542	0.988	0.177	0.972
% Solids-not-fat	P				0.038	0.727	0.050
D	G				0.604	0.983	0.691
Solids-not-fat	P					−0.103	0.987
E	G					0.550	0.944
% Total solids	P						0.052
F	G						0.211
Total solids	P						
G	G						

[a] Phenotypic correlations ≥ 0.25 and 0.32 are significant at the 0.05 and 0.01 levels of probability, respectively.

the percentages of solids-not-fat and of total solids could very well be independent of total milk production.

The genetic correlations between percentage of butterfat and percentage of solids-not-fat and percentage of total solids are positive and large for both herds. If linkage and selection are excluded as possibilities, this is strong evidence of the pleiotropic effects of genes which affect percentages of milk constituents. If a breeder were selecting for a higher percentage for one of the constituents, he would automatically increase the percentages of the other constituents. The genetic correlations are positive and large between fat and solids-not-fat and total solids and between solids-not-fat and total solids for both herds. The high correlations between total solids and the other two constituents of milk are to be expected, since total solids are made up of these two constituents. The genetic correlation between fat and solids-not-fat may be higher than anticipated. However, since fat and solids-not-fat both had a very high correlation with total solids, and both are constituents of total solids, they must have a high correlation with each other.

As expected, genetic correlations are generally higher than the phenotypic correlations. The phenotypic correlations that could be compared were very similar to those reported by other workers. Touchberry (*11*) reported a correlation of 0.871 between milk and butterfat for Holstein cows as compared to 0.751 for this study. The correlation between total solids percentage and butterfat percentage for this study was 0.839, while Overman and Sanman (*10*) report a correlation of 0.96. In another report, Black and Voris (*1*) found phenotypic correlations in Holstein cows of 0.90 between butterfat and total solids, 0.68 between butterfat and solids-not-fat, and 0.93 between total solids and solids-not-fat. The same correlations for this study were 0.82, 0.78, and 0.97, respectively.

The average milk, butterfat, solids-not-fat, and total solids production, as well as the percentages of these constituents of milk, for the 152 Holsteins and 140 Jersey cows in this study, are reported in Table 5.

The percentages for butterfat and solids-not-fat for the two breeds are very similar to those reported by White and Judkins (*13*). The Holstein herd was considerably higher than the Jersey herd in total milk production, as well as in the various constituents of milk studied, but was considerably lower in the percentage of the various constituents. Undoubtedly, this is a breed difference.

SUMMARY AND CONCLUSIONS

Two-day composite samples of milk were collected from 76 daughter-dam pairs of Holstein and 70 daughter-dam pairs of Jersey cows each month for the

TABLE 5

Average milk, butterfat, solids-not-fat, and total solids production for 305 days, 2X, M. E.

Breed	Cow No.	Milk lb.	Fat lb.	Fat %	SNF lb.	SNF %	T.S. lb.	T.S. %
Holstein	152	14,485	484	3.34	1,244	8.58	1,726	11.93
Jersey	140	6,847	372	5.45	656	9.54	1,027	14.99

first 305 days of lactation. The 76 daughters were from 8 different sires in the Holstein herd and the 70 daughters were from 8 different sires in the Jersey herd. The milk samples were analyzed for butterfat, solids-not-fat, and total solids.

The average production of the 152 Holstein cows in this study was 14,485 lb. of milk with 3.34% butterfat, 8.58% solids-not-fat, and 11.92% total solids. The average production for the 140 Jersey cows was 6,847 lb. of milk, with 5.45% butterfat, 9.54% solids-not-fat, and 14.99% total solids.

The heritability of each characteristic and the phenotypic and genetic correlations between the different characteristics were derived on an intra-sire basis. The Holstein herd had heritabilities of: milk, 0.30; butterfat, 0.30; per cent butterfat, 0.33; solids-not-fat, 0.35; per cent solids-not-fat, 0.34; total solids, 0.34, and per cent total solids, 0.38. The heritabilities for the Jersey herd for these seven characteristics were 0.26, 0.33, 0.33, 0.34, 0.36, 0.37, and 0.36, respectively. The various genetic correlations between the percentages of the constituents of milk were positive and relatively large, indicating that single genes affect several quantitative characteristics.

The very high genetic correlations between milk production and the production of the constituents of milk, as well as between the various constituents themselves, give strong evidence that there are single genes affecting all of these characteristics. The breeder, by selecting for any one of these characteristics, would automatically be selecting for the others, but with less pressure.

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INTERRELATIONSHIPS AMONG PLASMA 17-HYDROXYCORTICOSTEROID LEVELS, PLASMA PROTEIN-BOUND IODINE LEVELS, AND KETOSIS IN DAIRY CATTLE [1]

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Plasma levels of 17-hydroxycorticosteroids indicated adrenal-cortical activity and levels of plasma protein-bound iodine indicated thyroid activity in normal and ketotic cows. The 17-hydroxycorticosteroids averaged 5.09 μg. % for normal and 7.48 μg. % for ketotic cows, whereas the corresponding protein-bound iodine values were 4.22 μg. and 2.58 μg. %, respectively. These results suggest that ketosis might be caused by a relative adrenal-cortical deficiency induced by hypothyroidism. *Editor.*

Recent studies on ketosis in dairy cattle, as reviewed by Shaw *(22)* and by Armour Veterinary Laboratories *(1)*, give substantial support to the concept of ketosis as an endocrine disturbance. Hutyra and Marek *(7)* in 1926 postulated that adrenal-cortical exhaustion might be involved in ketosis in dairy cows. In 1947, Shaw *(21)* reported that extracts of the adrenal cortex were effective in treating ketosis. Reports *(12, 23, 26)* since then have affirmed the effectiveness of glucocorticoids and ACTH in treating ketosis. In a series of reports, Shaw and coworkers *(5, 24, 25, 27)* have presented results of a histopathologic study on 14 ketosis cows. The adrenals of these animals were enlarged, flabby, and showed degeneration of the cortex. Other conditions noted were regressive changes in the anterior pituitary gland, acute involution of the pancreas, involution of the thymolymphatic system, ulcers, nephrosis, and fatty changes in the liver. Fasting animals exhibited some of these same changes *(27)*, but did not show the adrenal and anterior pituitary gland changes. The authors suggested that these tissue changes were similar to the changes which Selye *(20)* reported as taking place during the alarm-reaction phase of the general adaptation syndrome, and led Hatziolos and Shaw *(5)* and Shaw *et al. (24)* to propose that bovine ketosis is due to an adrenal-cortical insufficiency, with possible anterior pituitary gland involvement.

Puntriano *(15)* studied the urinary glucocorticoid excretion in two normal lactating cows, two nonlactating, pregnant cows, and two ketotic cows, using the

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[1] Paper of the Journal Series, New Jersey Agricultural Experiment Station, Rutgers University, The State University of New Jersey, Department of Dairy Husbandry, New Brunswick. This work was supported in part by research grants from Swift and Co., Chicago, and Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Rahway, New Jersey.

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mouse-liver glycogen deposition method of Venning *et al. (28)*. The ketotic cows had a level of steroid excretion significantly lower than either of the two other groups, the nonlactating, pregnant cows having the highest level of excretion.

Robertson and Mixner *(19)* presented data which indicated that adrenal-cortical activity in dairy cattle declined post-partum, as measured by plasma levels of 17-hydroxycorticosteroids. Similarly, Lewis and Ralston *(10)* presented data which suggested a decline in thyroid activity post-partum, as measured by plasma levels of protein-bound iodine. These observations may be relevant to an understanding of the secretory status of both the adrenal cortex and the thyroid at time of onset of ketosis.

In this study an attempt was made to determine if ketosis in dairy cows was associated with an abnormal functioning of either the adrenal cortex or the thyroid, or both. A preliminary report of this work has already been made *(18)*.

METHODS AND PROCEDURES

Adrenal-cortical function was assessed by the measurement of the plasma levels of free 17-hydroxycorticosteroids (17-OH-CS), using the method of Robertson and Mixner *(19)*. The method of Brown *et al. (4)* was used for determining plasma levels of protein-bound iodine (PBI), used as the index of thyroid activity. Protein-free blood filtrates were prepared for blood glucose and blood acetone determinations by the Weichselbaum and Somogyi *(30)* method; whereas, the methods of Benedict *(2)* and Block and Bolling *(3)* were used for assay, respectively.

Blood samples were obtained from cows in private herds by one of the authors (W.W.B.). For each sample of blood from a ketotic cow, one was collected from a normal cow which most closely corresponded to the ketotic cow in age, breed, time since parturition, and level of milk production. Another series of 11 blood samples was obtained from ketotic cows, one to four weeks post-partum, for which no corresponding control blood samples were drawn.

RESULTS

Blood samples from ten ketotic cows and ten normal control cows were obtained for comparison in this study. Table 1 presents a summary of the data obtained. Statistical analysis of the data indicated that the ketotic cows had significantly higher levels of plasma 17-hydroxycorticosteroids and blood acetone, and significantly lower levels of plasma protein-bound iodine and blood sugar, than the control cows. Data on the blood samples from the 11 additional ketotic cows are presented (Table 2). A qualitative test for acetone was made on the plasma from these blood samples, using the Denco Acetone Test [4] (nitroprusside), the samples being rated on a 0 to 4+ scale. Higher levels of plasma 17-hydroxy-corticosteroids and lower levels of plasma protein-bound iodine were found in these samples, as compared with the control samples of the first series (Table 1).

[4] Manufactured by the Denver Chemical Mfg. Co., Inc., 163 Varick St., New York 13, N. Y.

TABLE 1

Composition of the blood of normal and ketotic cows with respect to glucose, acetone, plasma protein-bound iodine, and plasma 17-hydroxycorticosteroids

Cow pair No.	Days fresh		Blood glucose		Blood acetone		Plasma PBI		Plasma 17-OH-CS	
	N[b]	K[c]	N	K	N	K	N	K	N	K
			(mg. %)		*(mg. %)*		*(μg. %)*		*(μg. %)*	
1	11	14	39.7	19.3	2.4	14.6	3.07	1.94	6.12	9.41
2	22	42	44.6	22.6	1.0	19.6	3.98	2.01	6.17	6.17
3	25	10	39.6	24.9	3.4	28.8	4.23	1.82	6.04	7.13
4	60	60	46.3	32.9	4.2	9.3	3.87	2.46	5.53	7.89
5	51	47	45.3	29.6	1.8	17.4	3.72	2.81	2.93	6.94
6	33	−2[a]	40.4	26.5	2.4	15.6	3.22	2.43	2.68	8.89
7	18	8	38.1	17.5	3.2	23.7	3.06	2.86	4.18	7.63
8	15	19	42.0	24.7	2.6	18.4	3.58	2.33	4.81	7.72
9	17	−6[a]	40.8	36.4	1.4	24.3	3.48	2.14	8.40	10.51
10	35	7	44.4	33.3	3.3	12.8	2.98	5.01	4.01	2.46
Mean	28.7	19.9	42.1	26.8	2.6	18.5	4.22	2.58	5.09	7.48

[a] Days prior to parturition.
[b] N = normal control cows.
[c] K = ketosis cows.

TABLE 2

Composition of the blood of a supplementary group of ketotic cows only, with respect to glucose, acetone, plasma protein-bound iodine, and plasma 17-hydroxycorticosteroids

Cow No.	Blood glucose	Blood plasma acetone rating	Plasma PBI	Plasma 17-OH-CS
	(mg. %)	*(0 to 4 +)*	*(μg. %)*	*(μg. %)*
21LA5817	22.8	4 +	2.14	6.32
E4588	19.4	4 +	2.55	8.12
115630	26.7	4 +	2.83	5.78
F49353	29.1	1 +	2.66	7.65
E59606	39.5	1 +	3.34	5.71
21AC3052	37.9	2 +	2.28	6.87
C53559	30.5	2 +	2.77	10.13
F67458	18.7	4 +	1.63	8.29
E63727	17.5	4 +	2.49	7.21
4904	31.4	1 +	1.88	6.95
D70654	34.4	3 +	4.55	4.29
Mean	28.0	2.7 +	2.65	7.03

Because low-plasma protein-bound iodine values were found in the ketotic cows, it seemed important to define more carefully the normal changes in protein-bound iodine which occur around the time of parturition. Accordingly, plasma protein-bound iodine values were followed in ten cows from two to eight weeks post-partum (Table 3). A marked drop in plasma protein-bound iodine occurred at the time of parturition and in the 2-week period following. Return towards normal values was rather gradual.

DISCUSSION

If we assume that the low protein-bound iodine values in the ketosis animals reflect a state of hypothyroidism, results of this study are compatible with the theory that ketosis in dairy cattle involves a relative adrenal-cortical insufficiency

TABLE 3

Normal variation in plasma protein-bound iodine pre- and post-partum (mean of ten cows)

Time	Plasma PBI
	(μg. %)
Two weeks pre-partum	4.44
Partum	3.00
Two weeks post-partum	2.96
Four weeks post-partum	3.47
Eight weeks post-partum	4.08

as a consequence of the hypothyroidism. Hypothyroidism could affect the plasma steroid levels, either by decreasing rate of utilization, conjugation, degradation, and metabolism of the steroids by the liver and body tissues, or by affecting the basic rate of steroidogenesis in the adrenal cortex, through a lowered rate of adrenal-cortical metabolism. The degree to which either of these actions occurred could result in low, normal, or high levels of circulating steroids. This concept is supported by the work of Levin and Daughaday *(9)*, who demonstrated that there is a slow rate of disappearance of exogenous hydrocortisone from the blood of myxedematous people, indicating a slow rate of steroid degradation.

Hill *et al. (6)* have reported that many myxedematous patients failed to give an adequate adrenal-cortical response to ACTH. However, when the patients were treated with thyroid, restoring a normal metabolic condition, adrenal response to ACTH was also restored. The ketotic cow may be compared in this respect to the myxedematous person, in whom adequate blood levels of cortical hormone may not result in normal metabolic responses. Reece *(16)* reported that three of four ketotic cows responded favorably to 15 gm. of thyroprotein per day, suggestive of a possible thyroid involvement in ketosis. Other reports in the literature *(14)* indicate that there are profound interrelationships between thyroid and adrenal-cortical functioning.

With this background, it is now possible to understand how a low urinary excretion of glucocorticoids, as reported by Puntriano *(15)*, could occur in the presence of high plasma levels of glucocorticoids, as reported in this study. This would further support the idea that we are actually dealing with a relative adrenal-cortical insufficiency incident to the hypothyroidism.

Robertson *et al. (17)* have recently reported that liver function in ketotic cows is less than 50% of normal, as measured by the bromsulphaleindye–excretion test of Mixner and Robertson *(13)*. This is interesting in the light of the report by Klein *et al. (8)*, that patients with liver dysfunction conjugated endogenous or exogenous corticoids slowly, and that rate of removal of corticoids from the plasma was reduced. These circumstances could also result in an elevation of plasma glucocorticoids, as found in our study. It is interesting to speculate if the low liver activity was related to the suggested hypothyroidism in the ketotic cows.

It would seem important to evaluate the cause of the normal parturient and postparturient drop in the level of plasma protein-bound iodine. Lewis and Ralston *(10)* have presented evidence suggesting that it is related to a withdrawal

of plasma globulins and albumin into the colostrum milk. Other studies, Hill *et al.* *(6)*, have demonstrated that both ACTH and cortisone will decrease the I^{131} uptake gradient and the level of serum protein-bound iodine in normal people. This is interesting, because Robertson and Mixner *(19)* have shown that cows immediately preparturient have elevated plasma 17-hydroxycorticosteroid levels, as compared to postparturient cows. The possibility exists also that the depression in protein-bound iodine levels in ketotic cows found in this study was the result of the elevated corticoid levels. A nutritional approach to the cause of low protein-bound iodine values postparturient and in ketosis, would implicate the anterior pituitary gland and the thyrotrophic hormone. Vigue *(29)* and Marshak *(11)* have suggested that nutritional protein and energy deficiencies postpartum were related to the onset of ketosis.

Although determination of plasma 17-hydroxycorticosteroid levels may be quite helpful in gaining a better understanding of adrenal-cortical function in ketosis cows, its limitations must be realized. As pointed out, the level of thyroid functioning may influence steroid levels in the plasma through its direct and indirect metabolic effects. Other factors involved would include liver and kidney function, rate of steroid conjugation, biliary recirculation, adrenal-gonadal relationships, and the anterior pituitary–adrenal-cortical axis. The very complexity of these systems and the multiglandular involvements make interpretation of these results most difficult.

SUMMARY

Ten ketotic cows and ten normal cows, paired as to herd, breed, milk production level, time since parturition, and time of drawing and processing blood, were used in a study to relate adrenal-cortical and thyroid activity to the ketotic condition. Plasma level of 17-hydroxycorticosteroids was used as the index of adrenal-cortical activity, and thyroid activity was indicated by levels of plasma protein-bound iodine. Plasma 17-hydroxycorticosteroid values averaged 5.09 μg. % for the normal cows and 7.48 μg. % for the ketotic cows; whereas plasma protein-bound iodine values were 4.22 μg. % and 2.58 μg. %, respectively, for the normal and ketotic cows. In both instances, differences between mean values were highly significant statistically ($P < 0.01$). In another series of 11 ketotic cows (no paired control cows), the mean plasma 17-hydroxycorticosteroid values and the mean plasma protein-bound iodine values were 7.03 μg. %, and 2.65 μg. %, respectively. It will be noted that in the ketotic cows high 17-hydroxycorticosteroid values were associated with low protein-bound iodine values. This suggested that ketosis may be caused by a "relative" adrenal-cortical insufficiency induced by hypothyroidism.

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FREEZE-DRYING OF BOVINE SPERMATOZOA [1]

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Freeze-drying as a method of preserving spermatozoa was not successful. However, the experimental approaches have not been exhausted. *Editor.*

That bovine spermatozoa can be frozen, thawed, and then used for routine insemination is an established fact *(3, 4, 11, 14, 15, 19)*.

Freeze-drying has been successfully adapted to the preservation of bacteria and a great number of biological products. The literature is voluminous and for this reason the reader is referred to excellent reviews *(6, 9, 10)*. Polge and coworkers *(16)* were successful in freeze-drying fowl semen. Following immediate reconstitution, live cells were observed in every field, but after two hours storage no live cells were observed. This suggests that drying was not complete. These workers implied that studies on spermatozoa of other species were to be undertaken, but no further reports have appeared in the literature. Sherman *(17)* attempted to freeze-dry human spermatozoa by employing techniques used in preparation of materials for cytological studies. He reported only negative results. In this study, attempts were made to freeze-dry bovine spermatozoa.

EXPERIMENTAL PROCEDURE

In preliminary experiments, a modified Glick and Malmstrom freeze-drying apparatus *(7)* was used. The dimensions of the apparatus were as follows: trap capacity was 1,000 ml., distance between the trap and the surface of the drying semen was 45 cm.; the internal diameter of the connecting glass neck was 2 cm., and the neck had two 90-degree bends. The connection between the neck and the flask containing semen was made with a ground-glass joint. A Cenco-Pressovac-4 pump was used. The lowest pressure level obtained was 150 microns. This apparatus was found highly inefficient for dyring at temperatures below −30° C. For example, when the temperature of dehydration was −5° C., the time required for dehydration was eight hours.

Egg yolk citrate was used as the diluent. The egg yolk concentration was standardized at 23% by volume and the concentration of sodium citrate di-

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hydrate at 2.23%. An attempt was made to standardize the semen samples at 20×10^6 cells per ml. final concentration. Semen samples were diluted to one-half final concentration and placed in a refrigerator at from 4 to 6° C. Four to six hours later an equal volume of egg yolk citrate containing 15% glycerol by volume was added. The semen samples were then allowed to equilibrate with the glycerol for from 14 to 18 hours at this temperature. Semen for freeze-drying was placed in 200-ml. round-bottom flasks in 0.5 to 6.0 gm. quantities. The freezing rate was as follows *(11)*: +5° C. to −10° C., one degree every three minutes; −10° C. to −15° C. at one degree per minute. The temperature was then lowered rapidly by placing large amounts of dry ice in the alcohol both. A temperature of −70° C. to −75° C. was obtained within six to ten minutes following addition of a large amount of dry ice. The flask with the frozen semen was attached to the freeze-drying apparatus and the vacuum pump engaged. The frozen material was maintained at −65° C. to −70° C. until the pressure reached about 250 microns. At this point, the bath was removed and the material maintained in the frozen state by means of evaporative cooling. Drying temperatures were varied by submerging the semen-containing flask in a Dewar flask filled with alcohol at the desired temperature. The moisture trap was maintained at −75° C. to −78° C. by a dry ice alcohol slush.

No attempt was made to determine the degree of dryness in the preliminary study. In subsequent trials, the flasks were weighed empty, with semen in them, and again after drying. Thus, the weight of semen and the loss of weight following freeze-drying were determined. Percentage dehydration was calculated from total moisture determinations established by drying the glycerated control samples to constant weight in a vacuum oven, operated at 29 inches of vacuum and at from 110 to 115° C.

The freeze-drying apparatus was then modified to improve drying efficiency at the colder temperatures by reducing the distance between the surface of the frozen semen and the moisture trap. This apparatus resembles the one described by Glick and Malmstrom *(7)* in 1952, and more recently by Hansen and Robbins *(8)*. Also, a Welch Duo-Seal pump—1405 with an attached oil diffusion pump was used. Pressure was measured by a thermocouple gauge.[2] With this system, a pressure level of less than five microns was obtained within one hour.

Two drying surfaces were employed. In the first trial, semen was frozen in short bacteriological culture tubes (diameter one cm.). The average sample weight was 95 mg. The freeze-drying container had a capacity of five tubes, and the distance between the surface of semen and the cold trap was 4.5 cm. A drying temperature range of +20° C. to −35° C. was used. At the colder temperatures the drying time was 12 to 14 hours.

In the second trial, semen was placed on microscope slides in amounts comparable to ones used in routine microscope evaluations. The average weight of the semen samples prepared in this manner was 37 mg. Following the weighing of the semen-containing slide, a cover slip was placed on the semen surface

[2] National Research Corporation, Cambridge, Mass. Type 05-0100.

and the sample was frozen by being placed on the bottom of a household deep-freeze (−20° C.). The samples generally froze within four to five minutes. Then the slides were transferred rapidly onto the surface of a dry ice block and allowed to remain for from five to ten minutes. The cover slips were removed by placing a cold razor blade under them and prying upward. The error in semen weight introduced by the removal of the cover slip was less than one per cent. This procedure provided uniform layers of frozen semen, ranging in thickness from 0.2 to 0.05 mm. The distance between the surface of semen and the moisture trap was one cm. After this preliminary freezing, two slides were freeze-dried simultaneously. The moisture trap was cooled with a dry ice alcohol slush. Drying temperatures ranged from −45° C. to −70° C. This system was very efficient. Even at the colder drying temperatures, the drying time was only six to eight hours. Calculation of the percentage of dehydration was determined by the same method as described in the previous trial.

Rehydration was accomplished by adding cold distilled water, 0° C. to +5° C., to the dehydrated samples in amounts equivalent to the weight lost. The freeze-dried semen was rehydrated immediately after weighing, and no samples were stored in the dry state. The rehydrated samples were examined under a microscope and evaluations of the percentage of motile cells and of their rate of motility (1-10) were made. Differential staining with eosin-nigrosin was carried out on a number of samples in each of the trials *(18)*.

Frozen semen controls were maintained at the same temperature as the drying samples. They were examined at the same time as the rehydrated samples, or at the time when the temperature of drying was raised above freezing. All of the frozen semen controls, with the exception of those frozen on slides, were thawed in a water bath held at from +8 to +10° C. The controls frozen on the slides were thawed on a sterilizing oven with a surface temperature of from 85 to 90° C. The thawing time averaged nine seconds.

RESULTS

In a preliminary study, 33 samples of semen were freeze-dried, but the state of dryness was ascertained only by visual observation. All of these samples were dried at room temperature (23° C.). The vacuum pump was disengaged when the flask surface temperature reached room temperature. In routine freeze-drying such a procedure obtains a very high degree of dehydration. The data from this preliminary trial do not lend themselves to tabular presentation, since the degree of dehydration was not objectively determined. Twenty samples contained cells ranging in motility from few to possibly one per cent motile.

Table 1 presents results of the second trial. Moisture determinations were made on 65 freeze-dried semen samples, which were frozen in round-bottom flasks. These samples represented 11 bulls. The drying temperatures in this trial were maintained from −30° C. to 1° C. Since no obvious pattern in the relation of the drying temperature to the survival rate could be observed, the data are grouped according to the degree of dehydration.

TABLE 1

Visual evaluation of semen samples freeze-dried in round flasks, and the frozen controls

Per cent dehydration	No. of samples	Average reading	
		% motile	Motility rating
35-40	7	13.6	6.0
41-45	7	7.1	4.0
46-50	3	14.1	6.0
51-55	3	5.0	2.0
56-60	4	6.0	4.0
61-65	5	6.5	4.5
66-70	3	2.5	1.0
71-75	8	VFM[a]	
76-80	13	VFM[a]	
81-100	12	0	
Frozen controls		20.5	5.2

[a] VFM=Number of motile cells below one per cent.

In Table 2 the results of the third trial are presented. These data represent 60 semen samples from six bulls. These were frozen and dried in the short bacteriological culture tubes. As in the previous trial, no differences due to temperature of drying were observed. Consequently, the data are grouped according to the degree of dehydration. Frozen control samples, read at the same time as the rehydration samples, are also presented.

Table 3 contains data which represent samples of semen freeze-dried on microscope slides. Fifty-five samples representing five different bulls were used in this trial. Frozen semen controls were read immediately after freezing and then at the time when freeze-drying was complete. The data for the frozen control samples are presented in Table 4.

Table 5 represents the comparison of visual estimates of the percentage of motile cells to the percentage of live cells as determined by eosin-nigrosin staining. In no case was the visual evaulation higher than that obtained following the eosin-nigrosin staining.

TABLE 2

Visual evaluation of semen samples freeze-dried in short culture tubes, and the frozen controls

Per cent dehydration	No. of samples	Average reading	
		% motile	Motility rating
40-45	7	11.5	5.5
46-50	6	8.0	4.5
51-55	5	9.0	5.5
56-60	4	4.6	4.5
61-65	6	4.3	5.0
66-70	7	2.5	4.5
71-75	2	4.1	3.0
76-80	3	VFM[a]	
81-85	8	VFM[a]	
86-90	3	VFM[a]	
91-95	4	VFM[a]	
96-100	5	0	
Frozen controls		12.0	5.3

[a] VFM=Number of motile cells below one per cent.

TABLE 3

Visual evaluation of semen samples freeze-dried from microscope slides

Per cent dehydration	No. of samples	Average reading	
		% motile	Motility rating
30-35	2	17.5	6.5
36-40	3	18.0	6.0
41-45	5	6.7	5.0
46-50	3	11.0	5.3
51-55	3	8.3	5.7
56-60	4	7.5	5.7
61-65	3	2.5	4.5
66-70	2	3.5	4.5
71-100	30	0	

TABLE 4

Visual evaluation of frozen semen controls frozen on microscope slides under cover slips[c]

Glycerated semen		Frozen semen[a]		Frozen semen[b]	
% Motile	Motility	% Motile	Motility	% Motile	Motility
82.65	9.69	62.3	9.1	48.65	8.23

[a] Frozen semen thawed right after freezing.
[b] Frozen semen thawed at the time of reconstitution.
[c] These evaluations represent 26 samples.

TABLE 5

Visual observations of per cent motile cells as compared to eosin-nigrosin staining for live cells

No. of samples	Visual observation	Eosin-nigrosin staining
	(%)	(%)
15	0	5
8	0>1	8
6	1-5	16
7	6-10	26
5	11-15	29

Gross morphological examinations of stained and unstained samples did not reveal any induced abnormalities.

DISCUSSION

The purpose of this study was to attempt to evaluate freeze-drying as a method of preserving bovine spermatozoa. Results obtained in the preliminary study suggested that some spermatozoa withstand the stress of freeze-drying. Since no actual moisture determinations were made, it is difficult to know whether those few live cells actually represented live rehydrated cells or simply some cells from which moisture was not removed.

It is known *(14)* that storage of frozen spermatozoa at temperatures around −15° C. is highly deleterious; thus, it was felt that more successful results would be obtained by lowering the temperature of drying below −15° C.

From Table 2 it appears that some cells can withstand dehydrations as high as from 90 to 95%. As there were no stored frozen-dried samples, the question concerning live rehydrated spermatozoa following these high dehydration ranges remains unanswered. However, the thickness of the semen layer was considerable and, under these conditions, uniform drying cannot be expected unless dehydration is complete. With a relatively thick layer of semen, the degree of dehydration varies inversely with the distance from the surface.

Samples frozen under cover slips more closely approach the requirements for studying the effects of dehydration. The uniform, thin layer permits more uniform dehydration, coupled with an extremely large gain in efficiency. The highest dehydration level resulting in live sperm following reconstitution was 70.05%. This is of interest, since Billingham and Medewar *(2)* have shown than human skin will survive removal of not more than 75% of its content of water. They further make the statement that "there is no evidence that any mammalian tissue can withstand dehydration to, say, a residual water content of as little as five per cent."

Data (Table 4) illustrate that freezing of thin semen layers is compatible with survival. Two possible explanations can be offered: *(1)* the glycerated semen was of very good quality, and/or *(2)* the concept concerning freezing and thawing rates for semen of bulls may have to be re-examined. The relatively small drop in semen quality, when examinations were made immediately after freezing and at the end of individual trials, could be attributed to the cold drying temperatures employed during this study.

A puzzling picture is presented by Table 5. In all cases, staining revealed better survival than did visual examination. Since motile cells are definitely alive and the results of staining are questionable, we tend to favor the reliability of visual observations of livability. It is believed to be entirely possible that freeze-drying alters the staining reaction, especially since Mixner and Saroff *(12)* observed the effect of glycerol on the staining reaction.

Little attention was paid to the method of reconstitution, with the possible exception of using cold distilled water. It is unfortunate, but a search of the literature revealed little work on reconstitution methods *(10)*. Parkes *(13)* made it very clear that reconstitution may be important when he stated: "Much depends on the method of reconstituting it. In our experience of freeze-drying, that may be more important than the method of freezeing. It may be that if you haven't killed it by freezing and drying, you've killed it by your method of reconstitution." The possibility also exists that something besides water may be lost from the cell during the freeze-drying.

Cytological evidence *(1, 5)* shows that freeze-drying at temperatures below the eutectic point should produce few cyto-physiological changes. The drying temperature of −70° C. fulfills this requirement. However, in these experiments freeze-drying at −70° C., followed by the customary method of reconstitution, yielded negative results whenever the dehydration level exceeded 71%.

The possibility of a toxic change in the egg yolk diluent was tested; semen samples stored in freeze-dried reconstituted egg yolk showed good livability.

SUMMARY AND CONCLUSIONS

Data are presented relative to the applicability of freeze-drying as a method for preserving spermatozoa. Two hundred and thirteen samples were freeze-dried. Techniques used in these experiments gave negative results. It is felt that the data from the last trial are the most significant, for conditions were more reproducible. The possibility that freeze-drying can be a successful method for preservation of bovine spermatozoa is not excluded, because a great number of experimental approaches are still possible.

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EFFECT OF FEEDING LOW LEVELS OF DIETHYLSTILBESTROL ON ESTABLISHED LACTATION IN PAROUS DAIRY COWS

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Feeding diethylstilbestrol (DES) to lactating cows did not affect the amount of 4% FCM, percentage of SNF, efficiency of production, body weight, or utilization of TDN. The DES provided estrogenic activity equal to that found in forages. It is unlikely that increased production is due to estrogenic activity when cows are changed from dry to succulent forage. *Editor.*

Quantity of milk produced by lactating cows generally increases when the cows are changed from barn feeding to grazing lush pastures. Results of British studies reviewed by Pope *(18)* suggest that the galactopoietic qualities of young forage may be related to their estrogen content. Bartlett *et al. (2)* found estrogenic activity in grasses cut at an early-flowering stage. Cows grazing these grasses made moderate increases in milk production and in percentage of NFS (nonfatty solids). Similarly, increases in NFS have been obtained by injecting cows with diethylstilbestrol (DES), a synthetic estrogen *(7)*. Other investigators have fed or injected DES, with varying results.

In some studies, the DES appeared to stimulate increases in milk flow *(3, 21)*; whereas, in other studies, the treatment of lactating parous animals with DES either had little or no effect *(12, 19, 20)*, depressed *(13)*, or had inconsistent effects *(9)* on milk yields. However, Mixner *et al. (14)* noted that the degree of inhibition of milk secretion was proportional to the dosage of DES. In summary of the studies of effect of DES on milk production, the following statements appear justified: (*a*) treatment of dairy cows in established lactation with DES in excess of 4.0 mg. (or with other compounds having similar estrogenic activity) per day may be expected to increase NFS to the same extent as does the grazing of young forage; (*b*) levels of DES fed or injected have provided estrogenic activity that is several times greater than the estrogenic activity that would be obtained from forages, and (*c*) in established lactation the level of DES fed or injected, the level of milk production, and the stage of lactation appear to affect the lactation response to DES administration.

In this report, the term "estrogenic activity" refers to increases in weight of the uteri of immature or castrated mice and rats, from feeding or injecting naturally occurring or synthetic compounds.

The study reported here was initiated to determine the response of parous

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lactating dairy cows to administration *per os* of DES, at levels that would provide estrogenic activity within the general range of concentrations that have been reported as present in some high-quality forages.

EXPERIMENTAL PROCEDURE

Fifteen dairy cows that had passed lactation peaks, and would not be in mid-gestation before the experimental period terminated, were placed on a two-weeks' standardization, during which they were fed alfalfa hay and concentrates with low estrogenic potency. Subsequently, they were divided at random into three groups of five cows each.

The basal diets of the three groups during the 28-day experimental period were similar and consisted of green, leafy alfalfa hay (approximately U. S. No. 2 grade), plus a concentrate to supply the required TDN that was not consumed as hay. This diet fed during the standardization period was designated as the experimental control diet and the experimental Diets DES–1 and DES–50 were formed by feeding the cows 0.068 and 3.40 γ, respectively, of DES per lb. of feed. The DES supplement to Diets DES–1 and DES-50 was measured in capsules, and mixed with concentrate fed daily. During the first two weeks of the experimental period, the DES supplement was added twice daily; whereas, during the last two weeks, the quantity for the two feedings was combined, and given in the afternoon to reduce the time spent in preparing capsules.

The concentrate mixture included ground shelled corn, 39%; citrus pulp, 38%; cottonseed meal, 20%; steamed bonemeal, 2%, and salt, 1%. This concentrate contained 74.4% TDN and 10.4% DP (digestible protein).

The hay and concentrate were fed twice daily and the amounts fed and refused were recorded. In order to facilitate handling, the hay was chopped to approximately one to three inches in length, by passing it through a forage chopper.

Most of the cows received all of the alfalfa hay they would eat, which amounts supplied between 47 and 73% of the TDN consumed. When some of the forage allowance was refused, the quantity of concentrates fed was increased by an amount necessary to provide a near-constant level of TDN intake similar to that fed during the standardization period. The alfalfa hay fed contained 55.3% TDN and 9.2% DP. The mean quantities of TDN consumed during the experimental period, and expressed as percentages of the calculated requirements for body maintenance and milk production (at the standardization period levels) by diets, were: control, 108.2; DES–1, 110.2, and DES–50, 106.8. Digestibility of the feeds was determined by a conventional digestion trial.

Cows in all dietary groups were machine-milked twice daily; no clinical cases of mastitis were detected. Milk produced by each cow at each milking was weighed and the amount produced was recorded. Butterfat content of the milk from individual cows was measured by the Babcock method on two-day composites at the beginning and at the end of the experimental period, and

twice within the experimental period. Nonfat solids were determined by means of the lactometer *(8)* on one-day composite milk samples, at the intervals indicated for butterfat tests.

Three replications of the cows were housed in individual box stalls and had water available at all times. Cows in the other two replications were housed in individual stanchions and were watered twice daily. Stalls and stanchions were bedded with pine-wood shavings.

All experimental cows exercised from one to four hours daily in a paddock that had been scraped to remove vegetation. Normally, this period was between 9 A.M. and 1 P.M.

All cows in this experiment were weighed on the first three days of the standardization period, on the last three days of the standardization period, and on the three successive days at the end of the study.

RESULTS AND DISCUSSION

The average daily levels of 4% FCM and adjusted 4% FCM of cows fed the control diet and of those fed diets supplemented with 0.068 and 3.40 γ of DES per pound of air-dry feed consumed are given (Table 1). The adjusted production takes into account the differences in levels of milk production of individual cows during the standardization period. It shows that the estrogenic activity supplied in this diet did not have a significant effect on the levels of 4% FCM produced. These findings are similar to those reported by Spielman *et al. (19)*, when much higher levels of DES were injected intramuscularly, and the findings reported by Sykes *et al. (20)* for cows fed ten mg. of DES daily. Estrogenic activity supplied in Diets DES–1 and DES–50 is within the range reported for feeds *(5, 6, 11, 16, 17.)* Since the cows were fed TDN in excess of requirements that would have supported higher milk yields, the absence of a detectible effect of DES-feeding on milk yield indicates: (*a*) that levels of estrogenic activity similar to those reported for young forages *(9, 11, 18)* have no effect on milk yield, or (*b*) that the effects of plant estrogens on milk production are not in proportion to their estrogenic activity, as measured by increases in weight of the mouse uteri. It is recalled, however, that the highest levels of estrogenic activity of plants appear at or near the flowering stage *(11, 16)*. Conversely, the highest levels of milk production generally are ob-

TABLE 1

Average daily 4% FCM production associated with treatments by periods

Treatment groups	Production by periods		
	Standardization	Experimental	Experimental adjusted[a]
		(lb.)	
Control	30.70	28.74	26.28
DES–1	25.14	22.14	25.15
DES–50	28.76	25.48	24.93
L.S.D., P = 0.05	.....	.....	2.51

[a] Production during the experimental period, adjusted by covariance to take into account differences between groups during the standardization period.

tained on immature forage. This appears to support the hypothesis that plant estrogens do not materially affect milk yield.

Sometimes major changes in milk yield during an experiment are masked by counterbalancing increases and decreases in milk yield. Hence, a study was made of the weekly trends in milk production. The average daily milk yields at weekly intervals, associated with treatments, are shown (Figure 1). Cows

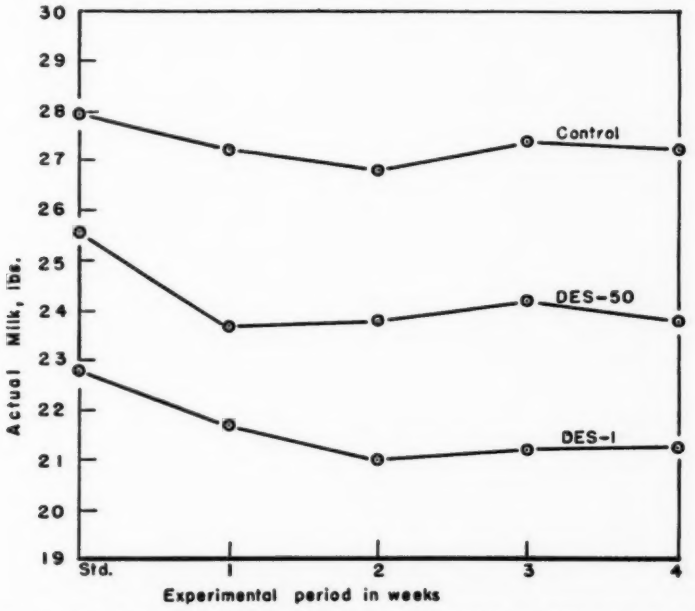

FIG. 1. Average daily milk production by treatments, during the final week of the standardization period and during the experimental period.

in all treatment groups produced less milk during the first week of the experiment than during the last week of the standardization period. Statistical treatment of these data revealed that the decreases were due to variations of individual cows and could not be considered as a true treatment response. Trends in milk production for the three groups were similar throughout the four-week experimental period.

The values representing gross utilization efficiency of consumed TDN for milk production *(15)*, during the experimental period, are given (Table 2). Gross efficiency was not affected significantly by the addition of DES to the diet. However, cows in all treatment groups were less efficient in converting TDN to milk during the experimental period than during the standardization period.

Percentages of NFS content of milk from cows in this study, by treatments,

TABLE 2

Gross efficiency of milk production by treatments and periods

Treatment group	Standardization	Period Experimental	Experimental adjusted[a]
		(%)	
Control	31.04	28.72	27.80
DES–1	27.50	24.50	26.53
DES–50	31.29	27.67	26.55
L.S.D., P = 0.05	.....	.....	2.12

[a] Adjusted by covariance, to take into account the differences between groups during the standardization period.

were: control, 9.23; DES–1, 9.41, and DES–50, 9.54. Arithmetical average levels of NFS were slightly higher in groups receiving DES than in the control. However, the small differences between treatment groups were not statistically significant. As pointed out earlier, this may indicate that there is no relationship between the so-called estrogenic activity of certain plant steroids and the effect of young forages on milk composition.

Body weight changes and efficiency of utilization of available TDN by these healthy cows were similar for all treatment groups. During the experimental period, the cows that received no DES in their diet gained an average of 28.4 lb. and those that received 0.068 γ and 3.40 γ of DES per lb. of feed gained 26.4 and 14.4 lb., respectively. The values representing efficiency of utilization of available TDN *(10)*, which include changes in body weight and estimated requirements for body maintenance and milk production, by treatments were: control, 114.1; DES–1, 113.9, and DES–50, 109.1. Thus, DES at 0.068 γ and 3.40 γ per lb. of feed did not produce a measurable effect on efficiency of feed utilization, such as has been reported for beef cattle *(1, 4)* fed ten mg. or more of DES daily. This difference in response of dairy and beef cattle may have been due to (*a*) the levels of the estrogen fed or, (*b*), the feed intake, which was limited for the dairy cows and unrestricted for the beef cattle.

Observations were continued into the postexperimental period, to determine whether the DES-feeding had a latent effect on milk yield. These postexperimental observations were nonconclusive, but indicated that there were no delayed effects from DES treatments.

SUMMARY

In a study involving 15 lactating cows, feeding diethylstilbestrol at levels of 0, 0.068, and 3.40 γ per lb. of feed did not affect significantly (*a*) the level of 4% FCM, (*b*) percentage of NFS in milk, (*c*) gross efficiency of milk production, (*d*) changes in body weight of the cows, or (*e*) efficiency of utilization of available TDN.

The levels of DES fed in this study provided estrogenic activity that is within the range of the estrogenic activity found in forages. Therefore, it is unlikely that the increase in quantity of milk and in NFS in milk, when cows change from dry feeds to succulent young forage, are due to estrogenic activity.

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CEREAL-GRAIN SCREENINGS PRODUCED IN THE PACIFIC NORTHWEST AS A FEED FOR LACTATING DAIRY COWS[1,2,3]

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Lactating cows were selected for age, breed, milk production, fat test, size, and stage of lactation and were fed rations in single reversal trials, in which cereal screenings replaced 30% of the other ingredients, except linseed oil meal in the concentrate ration. Their intakes of chopped alfalfa hay and silage were limited proportionately to body weight. The differences in four percent FCM production for the experimental and control groups were not statistically significant at the five per cent level. *Editor.*

Screenings are being used by dairymen and livestock feeders as a partial or a complete substitute for the concentrate ration. Screenings are used in grain-producing and surrounding regions because of their low cost.

There is good reason to question the use of screenings as a substitute for part or all of the concentrate ration because of: (*a*) their extreme variability in composition; (*b*) their frequently high crude fiber and ash content; (*c*) their frequently high content of weed-seeds, possible low nutritive value, and danger of weed propagation.

Dunsmore *(3)* fed various grades of wheat screenings to steers and dairy cattle. In these trials, recleaned elevator screenings proved to be equal to a good mixed meal for fattening steers. Recleaned elevator screenings proved valuable when composing one-third of the meal ration. Until the animals became accustomed to the change, however, screenings were unpalatable because of weed-seeds.

For lamb fattening, Linfield *(4)* found that a ration of lucerne and mixed grain or screenings, with or without beet pulp, was the most profitable of several rations fed. Linfield *(5, 6)* also conducted other feeding trials with sheep. Results indicated that screenings were the cheapest and most efficient grain ration for fattening lambs when fed with clover. The screenings were compared to wheat, oats, barley, and a mixture of these three.

Dunsmore *(3)* found that wheat screenings fed to lambs were a valuable addition to the meal ration when comprising one-third of the ration. When fed

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[2] These data were taken in part from a thesis presented by the senior author to the faculty of the State College of Washington, in partial fulfillment of the requirements for the Master of Science degree.

[3] The mixed cereal-grain screenings used in this trial were furnished by the Washington Co-operative Farmers' Association.

[4] Now at Cornell University, Ithaca, New York.

alone, wheat screenings had slightly less value than a mixed meal ration of good variety and quality.

Scott *et al. (9)* reported a TDN value of 58.4 for Canadian refuse screenings produced in the Pacific Northwest when fed alone, and 61.6 when fed with alfalfa. In this trial, sheep were used as experimental animals.

A study of the TDN value and weed-seed content of Pacific Northwest cereal-grain screenings produced in this area and used in this trial has been reported *(8)*. The present paper concerns the value of these screenings as a component of a concentrate ration for lactating dairy cows.

EXPERIMENTAL PROCEDURE

Sixteen lactating dairy cows were selected from the college herd as experimental animals. Cows were selected and grouped on the basis of age, breed, milk production, fat test, size, and stage of lactation. There were 12 Holsteins and four Jerseys. The cows were individually stanchioned, remaining in their stalls except for a cleaning period each day.

The feeding trial was of the single-reversal type with one-week preliminary and six-week experimental periods. There were two groups of eight animals and two rations.

Chopped alfalfa hay of average quality was fed at the rate of 1.2 lb. per 100 lb. of body weight, adjusted weekly for body weight changes. This system of hay feeding was used, instead of ad libitum feeding, to keep total roughage intake uniform. Grass silage of good quality was fed at the rate of three lb. per 100 lb. of body weight.

The constituents and amounts in the control and experimental concentrate rations are shown (Table 1).

TABLE 1

Concentrate rations fed to control and experimental cows

Ingredient	Control ration	Experimental ration
Mixed cereal-grain screenings		300
Barley	230	130
Ground cull peas	200	140
Ground whole corn	100	70
Ground oats	300	210
Linseed meal	50	60
Millrun	100	70
Steamed bonemeal	10	10
Iodized salt	10	10

In formulating the experimental ration, screenings were added to the control ration at a 30% level, with proportional adjustment of other ingredients. Protein was adjusted by increasing linseed meal, based on preliminary gross chemical analyses.

One pound of concentrates was fed for each three lb. of 4 per cent fat-corrected milk (FCM) produced.

The screenings furnished by a large mill in eastern Washington were a mixture of "sand," "air," and "cyclone" screenings, originating from barley, oats, corn, and wheat. They are considered representative of screenings produced in the Pacific Northwest. On a weight basis, the screenings used were a mixture of 1,237 lb. of barley screenings, 1,476 lb. of oat screenings, 1,522 lb. of wheat screenings, and 350 lb. of corn screenings. Mixed cereal–grain screenings were used (rather than screenings from one source), because this is the type of product that would be available for addition to concentrate rations, if dairymen or feed-mixers decided to use screenings.

Records of daily consumption of silage, hay, and grain, and milk produced, were kept. The cows were milked twice daily. Approximately 10 ml. of milk were composited at each milking, for weekly determinations of butterfat percentage.

Daily feed weigh-backs were saved from each cow and weighed each week. After weighing, a representative sample was taken and placed in a separate sack. At the end of each experimental period, a composite was taken for chemical analyses. Samples of silage, hay, and grain were taken periodically during the trial, for gross chemical analyses, and the average composition for each period was determined.

Body weights were taken on two consecutive days each week. The Babcock test was employed to determine the fat content of the milk samples. Standard A.O.A.C. methods *(1)* were used to analyze all of the feed and weigh-back samples. The effect of incorporating 30% screenings in the dairy concentrate ration was tested statistically, as recommended by Snedecor *(10)* and Cochran and Cox *(2)*.

In determining the total digestible nutrients (TDN) consumed, the coefficients obtained in digestion trials *(8)* were used for the alfalfa and screenings. The TDN in the other feeds consumed were calculated by applying digestion coefficients from Morrison's *(7)* tables to the gross chemical analyses of the feeds. The TDN in the weigh-backs were also estimated by means of Morrison's *(8)* tables, by basing the digestion coefficients on the fiber content of the weigh-backs.

RESULTS AND DISCUSSION

Table 2 shows the chemical composition, by experimental periods, of the feeds fed during the course of the experiment; it also lists the chemical composition of the mixed cereal–grain screenings. The screenings were rather light, chaffy, and high in fiber. Whereas additional linseed meal was added to the screenings—containing ration to equalize the protein content of the two concentrate rations—Table 2 shows that the control concentrate ration contained about two per cent more total crude protein. Apparently, the initial sample of screenings taken for protein determination was not representative of the entire lot. This discrepancy is not considered serious, since all cows were consuming sufficient protein to meet Morrison's *(7)* minimum requirements.

Another interesting fact shown (Table 2) is the wide variation in dry matter content in the silages used in Periods I and II. A new silo was opened at the

TABLE 2

Chemical composition and TDN of feeds fed

Feed	100% dry basis					As-fed basis	
	Crude protein	Ether extract	Crude fiber	Ash	Nitrogen-free extract	TDN	Dry matter
	(%)						
Period I							
Alfalfa hay	16.3	1.5	31.0	10.5	40.8	45.5[b]	93.2
Alfalfa-brome grass silage	14.1	3.3	32.6	10.2	39.8	12.2[a]	26.2
Control grain ration	16.0	4.7	8.5	5.0	66.0	74.7[a]	91.9
Grain ration + 30% mixed cereal-grain screenings	13.9	4.1	11.5	7.1	63.5	68.4[a]	91.7
Period II							
Alfalfa hay	14.4	1.6	33.3	9.1	41.8	46.0[b]	94.1
Alfalfa-brome grass silage	10.5	2.0	30.5	8.3	48.7	28.2[a]	50.6
Control grain ration	16.1	5.0	6.3	5.2	67.5	76.2[a]	92.5
Grain ration + 30% mixed cereal-grain screenings	14.1	4.3	11.5	7.8	62.4	68.6[a]	92.3
Mixed cereal-grain screenings	8.0	3.2	21.3	9.5	58.0	61.0[b]	92.5

[a] Calculated using coefficients from Morrison (*7*).
[b] Determined by digestion trial (*9*).

end of the second week of Period II. The dry-matter content of this silage was very high. When the cows were fed this silage at the rate of three lb. per 100 lb. of body weight, they consumed appreciably more dry matter and had greater refusals than during the first experimental period. Even though the amount of silage offered was then reduced somewhat, to decrease the amount of weigh-backs, cows still consumed more total dry matter from the silage than during Period I. Since both groups of cows reacted in the same manner to the increased dry matter of the silage, this departure from the experimental plan probably did not introduce appreciable error.

Table 3 shows that cows on the screenings ration produced 33.1 lb. four per cent FCM per cow daily, whereas those on the control ration produced 33.9 lb.

TABLE 3

Milk produced, feed consumed, TDN consumed and required, and body weight changes per cow daily

	Control ration	Screenings ration
Four per cent FCM (*lb.*)	33.9	33.1
Body weight gains (*lb.*)	−0.2	−0.4
Hay consumed (*lb.*) (as-fed basis)	13.9	13.9
Silage consumed (*lb.*) (as-fed basis)	31.6	31.6
Grain consumed (*lb.*) (as-fed basis)	11.7	11.4
TDN consumed (*lb.*)	20.3	19.4
TDN required[a] (*lb.*)	18.7	18.4
TDN consumed over requirements (*lb.*)	1.6	1.0

[a] Morrison's (*7*) minimum requirements.

Analysis of variance failed to show a significant difference in amounts of milk produced or body weight changes by animals on the two rations.

Figures 1 and 2 illustrate graphically the four per cent FCM produced daily by cows on the screenings and control rations. Figure 1 shows average production by treatment; Figure 2 shows production by treatments and groups. Figure 2 shows also that the production of Group 1 fell below that of Group 2, when Group 1 was switched from the control to the screenings ration and Group 2 from screenings to control. Differences in body weight gains between the two rations were nonsignificant. Thus, although the control ration had no statistically significant advantage over the screenings ration, either in milk production or body weight gains, it did, on the average, cause cows to produce at a slightly higher level (0.8 lb. four per cent FCM/cow/day) and gain more body weight (0.2 lb./cow/day).

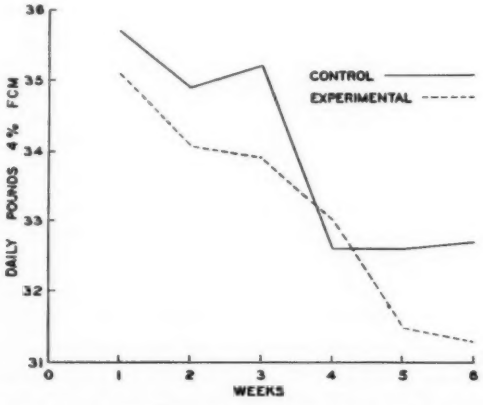

FIG. 1. Average four per cent FCM produced daily per cow, on the screenings and control rations.

Table 3 shows feed and TDN consumed per cow daily. It is interesting to note the close parallel in hay, silage, and grain consumed by treatments. The similarity is not unexpected, since both hay and silage were fed on a unit-weight basis. Nevertheless, both groups continued to eat silage at essentially the same rate, even during Period II, when silage refusals were great. Though hay and silage consumption by treatments was the same, the control cows consumed 0.9 lb. more TDN daily from concentrate, because they produced more milk and were fed according to production. Also, the calculated average TDN content of the control concentrate ration was 75.4% and that of the screenings ration, 68.5%. Morrison's *(7)* tables would indicate that the 0.9 lb. advantage in TDN intake should cause an advantage of two to three lb. in milk production. However, because cows at a high level of energy intake have less milk production response to added energy increments, it is believed that the small difference in production is due to the relatively high level of rationing. At a lower level of

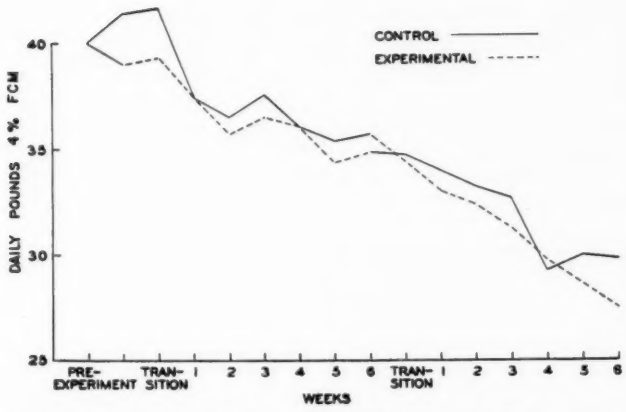

FIG. 2. Average, by periods, of four per cent FCM produced daily per cow, on screenings and control rations.

feeding, differences in milk production and body weight gains might have been more pronounced.

SUMMARY

A reversal-type feeding trial with six-week experimental and one-week preliminary periods was conducted with 16 lactating dairy cows. When screenings proportionately replaced 30% of other ingredients, except linseed oil meal, in the concentrate rations, the average daily yield of four per cent FCM was 33.1 lb., as compared with 33.9 lb. for the control rations. The difference was not statistically significant (five per cent level).

The cows on the control ration consumed more TDN than those on the screenings ration.

Animals on the control ration lost 0.2 lb./cow/day less body weight than those on the screenings ration, but the difference was not significant (five per cent level).

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STUDIES ON THE EXPERIMENTAL PRODUCTION AND PREVENTION OF BLOAT IN CATTLE. I. INFLUENCE OF INTRARUMEN ADMINISTRATION OF FRESH EGGWHITE ON PRODUCTION OF BLOAT[1]

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The role of protein, and quantitative changes in ruminal motility and eructation associated with bloat in cattle, were investigated. Intrarumen introduction of fresh eggwhite produced moderate bloat in cattle fed dehydrated alfalfa hay, either orally or by placing it in the rumen. Pressure in the rumen exceeded 15 mm. mercury above the atmosphere in two-thirds of the cases, when dehydrated alfalfa was fed before the intrarumen administration of eggwhite. Bloat in the rumen resulted from a stable foam which reduced frequency of eructation for 90 minutes after administration of eggwhite. Proteins may be important in the etiology of bloat from legumes. *Editor.*

For the past several years, it has been demonstrated repeatedly that the development of a stable foam within the rumen is an important factor in the etiology of bloat on legume forages (*7, 8, 12, 13*). Presumably, the foamy ingesta tends to block the cardia, interfering with eructation. Surface-active materials, such as methyl silicone, turpentine, or vegetable oils, have been shown to prevent development of bloat and to relieve this condition (*2, 5, 7, 8*).

A number of agents have been proposed as causative factors in developing a stable foam within the rumen. Quin (*12*) and Lindahl *et al.* (*11*) have reported that saponins may be an important factor in bloat on legumes. Hungate *et al.* (*7*) have demonstrated the presence of a particular type of slime-producing microorganism in the rumen, and they suggest that this contributes to formation of a stable foam. Johns (*8*) and Ferguson and Terry (*6*) have suggested the possibility that protein or protein-degradation products in the rumen are responsible, at least to some extent, for the formation of a frothy ingesta.

The present study was undertaken to investigate the possible role of protein in the development of bloat, and quantitatively to examine changes of ruminal motility and eructation associated with the presence of a mass of foamy ingesta within the rumen.

EXPERIMENTAL PROCEDURE

Influence of eggwhite administration on intrarumen pressure. Three dry Jersey cows, previously shown to be susceptible to bloat, were fed dehydrated,

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[1] In cooperation with the Animal Disease and Parasite Research Branch, Agricultural Research Service, U. S. Department of Agriculture.

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ground alfalfa ad libitum from 8 A.M. to 6 P.M. daily. The dry material was mixed with an equal part of water, to increase its palatability. The alfalfa, harvested in the prebud growth stage with a Gehl standard fly-wheel type forage harvester, was ground and dehydrated by a commercial operator, using conventional methods. Chemical analysis of the dehydrated meal by customary procedures gave the following values on a dry-matter basis: protein 30.9%, crude fiber 15.2%.

At intervals each animal received four liters of fresh eggwhite. This material, diluted with one liter of water and placed directly into the rumen with a stomach tube, was administered at approximately 10 A.M. (two hours after initiation of the day's feeding period). It was found that the eggwhite induced a prolonged increase of intrarumen pressure, such that the animals failed to eat for several hours following its administration. Thus, it was necessary to allow several days to elapse between drenches, for the cows to regain a fairly stable food intake. Alfalfa consumption of each animal for the two hours preceding the eggwhite drench, as well the total daily food and water intakes, was measured.

The degree of bloating was estimated with the tympanometer developed by Kleiber (*9*). Measurements were made at the first indication of a definite increase of intrarumen pressure, as manifested by a slight swelling of the animal's left flank, and repeated at 15-minute intervals until the pressure returned to normal levels. Since accurate measurements of slight increases in intrarumen pressure are not possible with the tympanometer, a slight puffiness in the left paralumbar fossa was assumed to be less than five mm. Hg above atmospheric pressure.

Effects of eggwhite administration on ruminal motility and eructation. Two adult cattle, a dry Jersey cow (897) and a Holstein steer (E802), were used in this phase of the investigation. Both animals were prepared in the following manner. A permanent ruminal fistula was established and fitted with an air-tight plug devised by Cole *et al.* (*3*). A method developed by Colvin *et al.* (*4*) was used to measure quantitatively the rate and extent of eructation. Briefly this technique is as follows: The trachea is transected; the severed ends are sutured to the skin and cannulated with plastic cannulas, respiration taking place through the cannula inserted into the posterior end of the severed trachea. The apparatus used for measuring the eructed ruminal gas consists of a mask fitted around the muzzle of the animal and made air-tight with a pneumatic seal. Tubing is led from the mask, and from the anterior tracheal cannula, to a spirometer. An inflatable plastic bag is inserted in the line ahead of the spirometer, in order to trap the gas expelled at each eructation and, incidentally, to establish the exact time of eructation, since the bag inflates markedly with each eructation. It is necessary to collect gas from the anterior tracheal cannula, as well as from the mask, because the majority of gas eructed is expelled from the former, presumably because it offers the pathway of least resistance.

In order to record, continuously, changes of intrarumen pressure, tubing is led from the rumen plug to a rubber tambour, which can be calibrated in centimeters of water above or below atmospheric pressure by means of a manom-

eter. The tambour activates an ink stylus, writing on a moving kymograph drum. This system also allows the continuous recording of ruminal contractions, as evidenced by sharp, positive deflections of ruminal gas pressure.

The procedure used in studying the effects of the intrarumen administration of the various materials on ruminal motility and eructation was as follows: Both animals were maintained on a constant daily intake of ground, dehydrated alfalfa, including the amount placed directly within the rumen during the trials, because it had been observed previously that the level of food intake of the preceding day had a marked effect on the results, particularly with respect to total ruminal gas production. Throughout the series of trials, therefore, Cow 897 received ten pounds of the dehydrated material each day, five pounds during the trial and five pounds at its conclusion. Steer E802 received 15 lb. of alfalfa daily, ten pounds during the trial, and five pounds at its conclusion. Both animals were fasted 12 to 16 hours before the next trial. Three or four days were allowed to elapse following the intrarumen administration of eggwhite before a new trial was started, to minimize any possible residual effect of the protein.

Near the conclusion of the 12- to 16-hour fast, the apparatus was placed on the animal and measurements were made of fasting ruminal motility and eructation for about 80 minutes. The rumen plug was removed and the test material placed into the rumen through the fistula, using a stiff plastic rod to mix the rumen contents, in vivo. After replacing the plug and apparatus, measurements were made of the time of eructation, with reference to the administration of the test material, amount of gas expelled at each eructation, ruminal motility, and level of ruminal pressure for a three-hour period. Since it required about ten minutes to place the material in the rumen, mix it with the rumen contents, and reconnect the apparatus, the timing of the trials actually began ten minutes after the first introduction of the test material into the rumen. In expressing the results, however, the time is referred back to the first introduction of the material into the rumen. At the conclusion of each trial, a sample of the rumen contents was removed through the fistula and its character noted. In trials where the intrarumen pressure remained above atmospheric pressure, free gas was drawn from the rumen, collected in a plastic bag, and measured with the spirometer.

Nine trials were conducted using Cow 897, six using Steer E802. Cow 897 received five pounds of dehydrated alfalfa and five liters of water in three trials; five pounds of alfalfa and four liters of fresh eggwhite diluted with one liter of water in three trials, and five pounds of alfalfa, four liters of eggwhite, and 80 ml. of vegetable oil in three trials. Duplicate trials were conducted with Steer E802. This animal received the same materials, except that the alfalfa was increased to ten pounds.

RESULTS

Influence of eggwhite administration on intrarumen pressure. Influence of the alfalfa meal and of the fresh eggwhite on the maximum resting intra-

TABLE 1

Influence of intrarumen administration of fresh eggwhite on intrarumen gas pressure of cows fed ground, dehydrated alfalfa

	Date	Daily alfalfa intake			Maximum intrarumen pressure [a]		
		Cow 47	Cow 54	Cow 58	Cow 47	Cow 54	Cow 58
		(*lb.*)			(*mm. Hg*)		
Feb.	16	15	14	10	0	0	0
	17	20	14	8	0	0	0
	18	8	15	10	0	0	0
	19	19	14	13	< 5	0	0
	20[b]	8(5)[c]	9(9)	7(4)	36	30	16
	21[b]	14(5)	7(5)	2(2)	11	13	7
	22	16	14	8	19	0	0
	23	20	18	11	< 5	0	0
	24[b]	10(7)	10(5)	5(4)	22	18	19
	25	18	13	11	< 5	0	0
	26	14	14	8	16	0	0
	27	15	17	14	< 5	0	0
	28[b]	13(9)	9(7)	4(3)	31	25	9
	29	19	14	10	< 5	0	0
March	1	14	17	8	< 5	0	0
	2	18	13	12	< 5	0	0

[a] Determinations made with a tympanometer (*9*), immediately following a rumen contraction.

[b] Administered four liters of fresh eggwhite via stomach tube.

[c] Alfalfa intake during the two hours preceding eggwhite administration.

rumen pressures of the three animals is presented (Table 1). It can be observed that the pressures attained are much greater, in general, following administration of eggwhite. An exception is in Cow 47, in which the intrarumen pressure rose to only 11 mm. Hg above atmospheric pressure on the 21st of February, and on the 22nd and 26th, when the eggwhite was not given, reached 19 and 16 mm. Hg, respectively. These variations may be explained by the fact that total alfalfa consumption for the 24 hours preceding eggwhite administration on the 21st of February amounted to only eight pounds. (A considerable amount of fermentable material must be present in the rumen before the eggwhite will induce bloat.) A similar reduction of food intake can be noted for Cows 54 and 58, which consumed only five pounds of alfalfa meal during the 24 hours preceding the eggwhite drench on the 21st of February. The eggwhite-induced bloat in these two animals for this day was also less severe, in comparison with that produced on other days.

Cows 54 and 58 did not bloat on the alfalfa meal alone, but the eggwhite produced a rise of intrarumen pressure every time it was administered (although in the latter animal this rise was not marked). Daily consumption of alfalfa by Cow 58 was considerably less than that of the other two animals and, again, probably explains the much less severe bloat encountered in this particular animal. Reduction of food intake following administration of eggwhite could be attributed to the resulting bloat, which lasted for a number of hours after drenching. Figure 1 is a typical curve of the change of intrarumen pressure with time, following administration of eggwhite (Cow 54, February 20, chosen as an example). Although intrarumen pressures attained a maximal level about one and one-half hours after eggwhite administration, the pressure remained con-

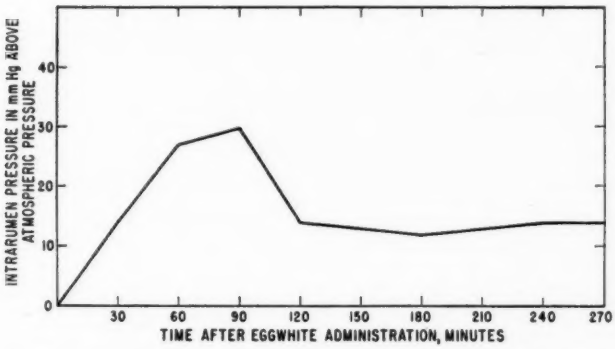

FIG. 1. Change of intrarumen gas pressure with time, following administration of fresh eggwhite to Cow 54 on February 20, 1956.

sistently above normal for several hours. In this particular case, the pressure was still 15 mm. Hg above atmospheric pressure six hours after the eggwhite drench. As soon as the pressure exceeded ten mm. Hg, the animals refused the alfalfa and would not resume eating until the pressure again dropped below this level.

Although the bloat induced by the eggwhite was not severe in these trials, a definite increase of intrarumen pressure occurred in every case following its administration. The alfalfa meal, even when mixed with an equivalent amount of water, was not palatable, limiting food intake and thus the amount of fermentable material available for the production of ruminal gas. In the presence of greater amounts of ingested materials, it is probable that eggwhite administration would have resulted in greater increases of intrarumen pressure.

Effects of eggwhite administration on ruminal motility and eructation. In order to simplify the presentation of results, data obtained for Steer E802 are not tabulated in this report. However, with the exception of a greater total ruminal gas production associated with the larger amount of alfalfa administered, the changes of intrarumen pressure, eructation, and ruminal motility follow the same trends as those found in Cow 897. (See data in Table 2 and Figure 2).

Table 2 lists the average resting intrarumen pressure, number of ruminal contractions, amount of gas eructed, and average interval between eructations for each individual trial, during consecutive 20-minute periods after administration of the test materials. Individual trial results are presented, to show variations which occur in triplicate trials under presumably identical conditions. The fasting figures are the average levels attained during the last 20-minute period of six 80-minute trials, conducted after a 12- to 16-hour fasting period. The resting intrarumen pressures were calculated by averaging the pressures measured while the rumen was at rest (not contracting) at several points during each 20-minute period.

The means of the values calculated from the triplicate trials are presented [Figures 2(a), (b), (c), and (d)], to depict more clearly the changes of eructa-

TABLE 2

Comparative effects of intrarumen administration of alfalfa,[a] alfalfa and fresh eggwhite, and alfalfa, eggwhite, and vegetable oil, on ruminal motility and eructation of Cow 897

(A=resting intrarumen pressure; B=number of rumen contractions; C=amount of gas eructed; D=average interval between contractions)

Interval (min.) after intrarumen administration	Trial No.	Material administered: Alfalfa (5 lb.) A	B	C	D	Alfalfa and eggwhite (5 lb.) (4 l.) A	B	C	D	Alfalfa, eggwhite, and oil (5 lb.) (4 l.) (80 ml.) A	B	C	D
		(*mm. Hg*)		(*l.*)	(*sec.*)	(*mm. Hg*)		(*l.*)	(*sec.*)	(*mm. Hg*)		(*l.*)	(*sec.*)
Fast	I	−4.4[b]	22	3.0	240	−4.4	22	3.0	240	−4.4	22	3.0	240
	II												
	III												
10-30	I	0.7[c]	35	.....	....	−1.5	....	5.7	245	−2.5	25	7.5	142
	II	−4.9	28	7.0	142	−1.8	31	6.4	198	−1.8	36	11.9	108
	III	−4.0	25	7.9	147	0.0	27	2.7	387	−2.6	32	6.9	179
30-50	I	−0.4	30	11.1	161	.....	....	....	.....	−2.1	27	7.6	159
	II	−3.8	26	6.1	186	−0.3	29	3.3	625	−2.8	30	6.6	169
	III	−2.3	27	10.4	152	1.9	30	8.3	253	−1.2	28	12.0	138
50-70	I	−0.7	26	11.6	146	.....	....	.....	.....	−1.6	24	7.8	125
	II	−3.2	23	5.4	196	0.9	28	7.0	204	−1.3	30	9.6	149
	III	−1.6	27	14.1	120	2.8	30	8.8	245	−0.7	29	14.4	139
70-90	I	−0.3	27	.....	.....	4.3	27	13.7	142	−1.0	27	7.4	111
	II	−2.6	24	5.8	212	2.8	26	5.0	313	−0.3	31	13.8	118
	III	−1.6	25	12.7	113	4.9	27	16.6	142	−1.4	26	13.1	132
90-110	I	−0.5	26	7.5	173	5.3	27	6.2	208	−0.9	26	7.4	111
	II	−1.1	27	8.3	125	5.5	25	8.5	164	−1.0	28	10.3	165
	III	−1.3	25	6.5	265	4.8	32	12.2	152	−2.6	23	13.6	152
110-130	I	0.0	25	9.6	133	8.1	28	11.6	94	−1.0	25	7.3	138
	II	−1.2	25	7.3	214	7.6	29	12.4	169	−0.7	29	11.1	113
	III	−1.0	26	12.8	129	6.6	33	12.2	147	−2.7	26	9.1	167
130-150	I	.....	....	6.0	138	9.7	24	.....	.....	−1.2	26	9.6	105
	II	−2.2	22	7.7	182	9.4	27	11.4	196	−1.2	35	13.7	116
	III	−1.0	25	8.3	209	7.6	27	13.6	128	−3.1	26	10.9	138
150-170	I	.....	....	.....	.....	.....	....	.....	.....	−2.0	27	10.0	104
	I	−2.1	27	5.0	313	10.7	30	11.3	162	−2.4	24	7.5	191
	III	−1.2	25	6.9	201	5.7	31	7.1	223	−2.8	25	8.2	147
170-190	I	.....	....	.....	.....	.....	....	.....	.....	−1.9	28	10.1	116
	II	−1.8	23	6.3	239	12.4	32	8.8	232	−2.1	27	9.0	180
	III	−0.4	21	6.5	174	6.8	29	8.6	208	−2.4	23	8.1	198
Total gas eructed (mean)				76.2				84.9				91.7	

[a] Ground, dehydrated alfalfa, harvested in the one-per cent bud growth stage.
[b] Below atmospheric pressure.
[c] Above atmospheric pressure.

tion and motility associated with development of the eggwhite-induced bloat. Although differences are not significant statistically, for the most part because of the few trials, consistent changes of intrarumen pressure, ruminal motility, and eructation were associated with administration of eggwhite in all three trials.

In addition to the progressive rise of intrarumen pressure, prevented by the simultaneous administration of vegetable oil [Figure 2(a)], the most consistent change following eggwhite administration was the interference of eructation for approximately 90 minutes [Figure 2(c) and (d)]. Primarily, this interference involved a reduction of frequency of eructation [Figure 2(d)], the

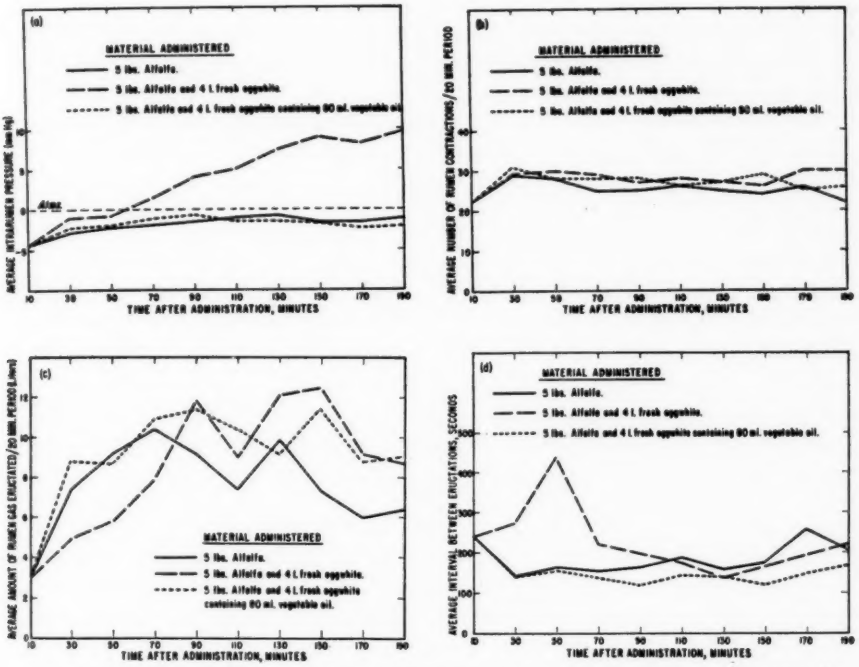
(a)
MATERIAL ADMINISTERED
5 lbs. Alfalfa.
5 lbs. Alfalfa and 4 l. fresh eggwhite.
5 lbs. Alfalfa and 4 l. fresh eggwhite containing 90 ml. vegetable oil.
AVERAGE INTRARUMEN PRESSURE (mm Hg)
TIME AFTER ADMINISTRATION, MINUTES
(b)
MATERIAL ADMINISTERED
5 lbs. Alfalfa.
5 lbs. Alfalfa and 4 l. fresh eggwhite.
5 lbs. Alfalfa and 4 l. fresh eggwhite containing 90 ml. vegetable oil.
AVERAGE NUMBER OF RUMEN CONTRACTIONS/80 MIN. PERIOD
TIME AFTER ADMINISTRATION, MINUTES
(c)
MATERIAL ADMINISTERED
5 lbs. Alfalfa.
5 lbs. Alfalfa and 4 l. fresh eggwhite.
5 lbs. Alfalfa and 4 l. fresh eggwhite containing 90 ml. vegetable oil.
AVERAGE AMOUNT OF RUMEN GAS ERUCTATED/80 MIN. PERIOD (L/min)
TIME AFTER ADMINISTRATION, MINUTES
(d)
MATERIAL ADMINISTERED
5 lbs. Alfalfa.
5 lbs. Alfalfa and 4 l. fresh eggwhite.
5 lbs. Alfalfa and 4 l. fresh eggwhite containing 90 ml. vegetable oil.
AVERAGE INTERVAL BETWEEN ERUCTATIONS, SECONDS
TIME AFTER ADMINISTRATION, MINUTES

FIG. 2. Average variations of intrarumen pressure, eructation, and ruminal motility of Cow 897, following intrarumen administration of alfalfa, alfalfa and fresh eggwhite, and alfalfa, eggwhite, and vegetable oil.

average amount of gas released at each eructation being greater in comparison to that observed with alfalfa alone or when oil was added to the eggwhite. As a result of decrease in frequency of eructation, the total gas expelled for the first 80 minutes was reduced, accounting for the accumulation of gas in the rumen and the resultant rise of intrarumen pressure. Thereafter, however, efficiency of eructation was approximately the same in all trials, in spite of the continued development of bloat following use of eggwhite.

Average levels of gas eructed during the entire three hours of the trials amounted to 76.2 l. following alfalfa, 84.9 l. following alfalfa plus eggwhite, and 91.7 l. following alfalfa, eggwhite, and oil (Table 2). An average of 9.8 l. of gas was collected from the rumen at the conclusion of the trials in which alfalfa and eggwhite were administered. Thus, the total gas production appeared to be greater following eggwhite (94.7 l.), compared to that following eggwhite and oil (91.7 l.). Further, the eggwhite resulted in the accumulation of a large mass of foamy ingesta, in which considerable gas appeared to be trapped. Thus, it would appear that the addition of vegetable oil inhibited total ruminal gas formation. A more logical explanation, in view of the relatively small amount of oil used, is that the mass of foam has reduced the passage of gas, particularly

carbon dioxide, through the rumen wall. Kleiber (*10*) has estimated that diffusion of gas through the rumen wall exceeds three l. per hour, a rate which could well account for the apparent decrease of total gas production associated with administration of oil.

The total ruminal contration rate remained essentially the same in all trials [Figure 2(b)], except for the increase over the fasting level associated with the introduction of material into the rumen.

As noted above, the eggwhite produced a large mass of stable foam which nearly completely filled the rumen. At the conclusion of the trials, when the rumen plug was removed, this foamy mass spouted from the fistula as it expanded, forming a column several feet in height. In contrast, the ingesta collected from the rumen following the administration of alfalfa alone, or alfalfa, eggwhite, and oil, was a soupy fluid containing only a few large bubbles of foam.

DISCUSSION

The data demonstrate that the administration of dehydrated alfalfa and fresh eggwhite produces a definite increase of intrarumen pressure by inducing the formation of a stable foam, which partially interferes with eructation. Ferguson and Terry (*6*) have reported that the intrarumen administration of egg albumin into a single sheep produced bloat when an extra supply of gas was made available. In this case, $NaHCO_3$ was used. In another series of trials, however, lucerne juice, egg albumin, and bicarbonate were ineffective. We have found that a considerable quantity of fermentable material (alfalfa) must be available before eggwhite will induce bloat. Intrarumen administration of four liters of fresh eggwhite alone into Cow 897 had no effect upon the intrarumen pressure.

In the trials utilizing Cow 897 and Steer E802, the various test materials were placed directly within the rumen. The question arose as to whether this procedure, in comparison with oral feeding, might have an effect on the results, particularly in view of the suggested influence of saliva on bloat (*13*). This question was tested, using Steer E802. A series of trials were conducted in which ten pounds of the dehydrated alfalfa either was fed orally or placed in the rumen. Determinations of intrarumen pressure, eructation, and ruminal motility revealed no differences attributable to the mode of administering the test substance, except for a slight delay of gas production, associated with the time required for the material placed in the rumen to become mixed with the rumen contents.

The fact that fresh eggwhite will induce frothing, reduce efficiency of eructation, and thus produce bloat does not prove that the causative factor of legumes responsible for bloat is water-soluble protein. In fact, Ferguson and Terry (*6*) have subjected alfalfa extracts to heat (60° C.) treatment with 75% alcohol, and passage through cation and anion exchange resins, without reducting their ability to produce bloat. These facts would suggest that the plant protein is not important in the etiology of bloat. On the other hand, we have observed that commercial dehydration procedures destroy the bloat-producing ability of freshly chopped alfalfa (*1*), a finding which would suggest that protein

may be of importance, assuming that the major effect of dehydration on plant constituents is a denaturation of water-soluble protein. It is suggested that the protein constituents of legumes may be an important factor in the etiology of bloat in cattle.

SUMMARY

Studies using five head of cattle show that intrarumen introduction of fresh eggwhite produces moderate bloat in cattle given ground, dehydrated alfalfa, either by oral feeding or by placing it directly into the rumen. In eight of 12 instances in which the dehydrated alfalfa was fed preceding intrarumen administration of eggwhite, the ruminal pressure exceeded 15 mm. Hg above atmospheric pressure, as measured with a tympanometer. Such bloat results from the formation of a stable foam within the rumen, which reduces the frequency of eructation for the first 90 minutes following administration of the eggwhite. It is suggested that water-soluble plant protein may be an important factor in the etiology of bloat on legumes.

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DIGESTIBILITY OF THE TOP AND BOTTOM PORTIONS OF THE ALFALFA PLANT, AS ESTIMATED FROM SMALL, RANDOMLY COLLECTED SAMPLES OF FECES [1]

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Digestibility of the top and bottom portions of the alfalfa plant was estimated from the concentration of plant chromogen in samples of feces collected at various times during the day. Estimates derived from samples collected at 6 P.M. were significantly lower than D.M. digestion coefficients determined by the conventional method. Coefficients predicted from feces voided at 6 A.M., 12 noon, and 12 midnight did not differ significantly from those determined by the total collection method. The excretion of chromogen was more uniform for animals receiving the top portion of the plant than for those receiving the bottom portion. The digestibility of the bottom part of the plant decreased 0.6% per day and it was only 87% as digestible as the top part which did not change markedly in digestibility during the trial. *Editor.*

Results of Reid *et al. (8)* from a limited experiment suggested it was possible to predict accurately the digestibility of pasture forage from the analysis of small, randomly collected samples of feces. Further work by Hardison and Reid *(5)* showed that grab-sampling of feces for the estimation of dry-matter digestibility was feasible. More recently, Soni and coworkers *(12)* have shown that there is no diurnal variation in the dry-matter digestion coefficients of grazing sheep and heifers, as estimated by the fecal chromogen or fecal nitrogen method. Thus, it is indicated that accurate results can be obtained from feces collected anytime during a 24-hour period. The results of Reid *et al. (8)* and also those of Soni *et al. (12)* were based on fecal samples collected during only the daylight hours.

This work was undertaken to study further the accuracy of dry-matter digestibility estimates, derived from the concentration of plant chromogen, in small samples of feces collected at various times during the entire day, and to compare the digestibility of the top and bottom portions of the alfalfa plant. Preliminary results of this work appear in abstract form elsewhere *(3)*. Also,

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the composition of the two portions of forage has been published in abstract form *(4)*.

EXPERIMENTAL PROCEDURE

Eight Holstein heifers, which had been on pasture for about two weeks before the start of the experiment, were used. The animals were divided into two comparable groups on the basis of age and weight; one group was fed the top portion of the alfalfa plant and the second group, the bottom portion of the plant. The alfalfa was in the prebloom stage. A sufficient quantity of forage was cut each morning to serve as feed for that particular day. A specially designed power-mower was used to cut the top and bottom portions of the forage. The animals were fed twice daily those amounts of forage consumed with a minimum amount of wastage. Consumption ranged from about 45 to 60 lb. per head, with the bottom half of the plant being consumed in essentially the same amounts as the top half.

Following a four-day preliminary period, feces were totally collected for a period of seven days. In addition, partial fecal samples were collected from each animal at 6 A.M., 12 noon, 6 P.M., and 12 midnight on each of the seven days. Dry-matter digestibility estimates were obtained from the partial samples by the plant-chromogen technique. Extraction of the pigments from the fecal samples was carried out essentially as outlined by Reid *et al. (9)*. Chromogen concentration was determined by the method of Reid *et al. (8)*, after the spectrophotometer used in this laboratory had been calibrated against Reid's instrument. In a preliminary investigation, it had been found that the modified chromogen method suggested by Smart *et al. (10)* did not give results comparable to the method of Reid *et al. (8)*.

RESULTS AND DISCUSSION

Excretion of the plant chromogen by the two groups of animals is shown graphically (Figure 1). In general, variation in excretion of the indicator was greater in the animals receiving the bottom portion of the alfalfa plant than in those fed the top portion. Excretion of the indicator (expressed as a per cent of the mean excretion) varied from about 85 to 113% for the "bottom" animals and from 92 to 105% for the "top" animals. Feces of the "bottom" animals, collected at 6 A.M. and 12 noon, were consistently higher in concentration of the indicator than were feces collected at 6 P.M. and 12 midnight. A similar but inconsistent trend was noted for the "top" animals. A similar pattern in the excretion of Cr_2O_3 by hand-fed animals was observed by Hardison and Reid *(6)* and by Kane *et al. (7)*, as reported by Hardison and Reid *(6)*. Excretion curves for Cr_2O_3 of the same general form were obtained by Hardison *et al. (2)*, for animals administered the indicator once daily. The excretion pattern of Cr_2O_3 in the free-grazing animal was directly opposite to that just discussed *(6, 11)*. That is, the feces voided by grazing animals at 6 P.M. and 12 midnight contained higher concentrations of the indicator than those produced at 6 A.M. and 12 noon. Apparently the difference was due, in part at least, to the difference in time of day when feed was consumed.

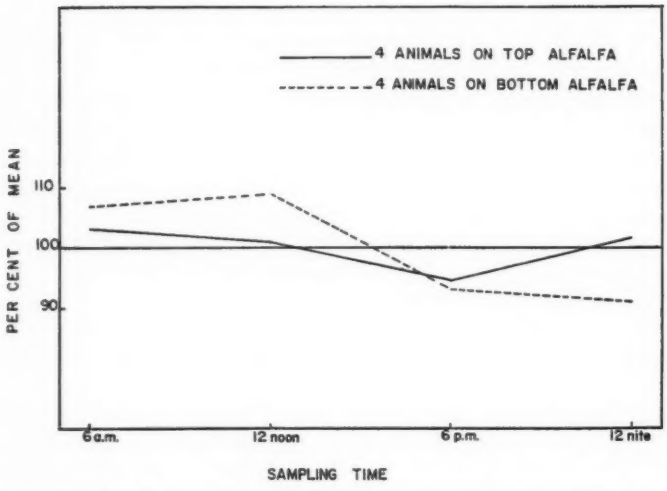

FIG. 1. Excretion patterns of plant chromogen, expressed as per cent of mean.

Digestion coefficients of dry matter derived from the concentration of plant chromogen in the partial fecal samples are summarized (Table 1). These values are quite uniform, and they compare very closely with the coefficients determined by the total collection method. The mean digestibility estimates determined by the chromogen technique and by the conventional method were 70.1 and 70.2%, respectively.

Statistical analysis revealed no significant difference in digestibility estimates derived from fecal samples collected during the day (6 A.M. and 12 noon). Neither did estimates derived from fecal samples collected at 6 P.M. differ significantly from estimates obtained from feces collected at 12 midnight. However, estimates derived from feces collected during the day were significantly different (one-per cent level) from estimates obtained from feces collected during the night (6 P.M. and 12 midnight), even though there was a difference of only 0.8 of a percentage point between the average of the day and night estimates. This is not in agreement with the data of Soni *et al. (12)*, which showed no essential difference between coefficients estimated from feces collected at 5 P.M. on the one hand, and 8 A.M. or 1 P.M. on the other. Bradley *et al. (1)* observed no difference in dry-matter digestion coefficients estimated from fecal samples collected at 6 A.M., 12 noon, 6 P.M., and 12 midnight, when sheep were grazing fescue pasture; however, on a ladino-alfalfa pasture, fecal samples obtained at 6 A.M. and 12 midnight gave lower coefficients than feces voided at 6 P.M. and 12 noon.

Further analysis of the data showed that dry-matter digestion coefficients estimated from small fecal samples collected at 6 A.M., 12 noon, and 12 midnight did not differ significantly from coefficients determined by the conventional method. On the other hand, feces collected at 6 P.M. gave coefficients significantly lower (one-per cent level) than those obtained by the conventional method. Thus,

TABLE 1

Digestion coefficient of dry matter estimated from the concentration of fecal chromogen

Day	Sampling time	Animals receiving top alfalfa				Animals receiving bottom alfalfa				Av. for all animals
		1	2	6	7	3	4	5	8	
		(%)								
	6 A.M.	74.6	76.5	75.6	75.6	68.4	68.0	66.4	68.5	71.7
I	12 Noon	74.6	73.4	76.1	73.3	66.9	67.8	68.4	67.7	71.0
	6 P.M.	75.1	75.7	74.9	73.7	65.9	66.7	67.0	67.1	70.8
	12 Nite	76.5	72.7	76.1	75.8	66.2	67.1	66.7	66.0	70.9
	6 A.M.	76.7	76.6	75.6	76.6	66.8	66.5	62.3	65.4	70.9
II	12 Noon	77.2	75.1	76.0	76.6	67.9	67.5	64.8	67.5	71.6
	6 P.M.	74.2	75.5	74.2	75.6	62.6	68.3	64.1	64.8	70.0
	12 Nite	74.4	76.4	75.9	75.5	63.9	65.0	64.5	63.9	70.0
	6 A.M.	76.2	75.0	76.5	76.5	68.5	69.7	63.7	66.7	71.6
III	12 Noon	75.6	76.0	76.4	75.5	66.0	69.5	67.6	64.8	71.5
	6 P.M.	74.8	75.9	76.3	76.6	66.7	66.4	64.3	64.2	70.7
	12 Nite	75.8	75.9	75.9	76.1	66.1	65.6	65.3	64.6	70.7
	6 A.M.	76.0	74.3	76.1	75.8	65.4	64.9	62.3	67.5	70.3
IV	12 Noon	75.5	76.1	75.8	76.3	64.0	65.9	63.9	66.2	70.5
	6 P.M.	76.1	75.3	75.8	75.5	62.3	62.6	64.0	65.2	69.6
	12 Nite	75.2	75.5	75.2	74.7	62.3	66.5	62.1	65.6	69.7
	6 A.M.	75.0	74.4	75.3	74.3	63.1	63.4	65.3	66.3	69.7
V	12 Noon	74.0	73.6	76.3	74.5	63.5	65.9	62.8	64.8	69.5
	6 P.M.	74.4	74.8	75.0	74.1	62.6	62.4	62.2	64.7	68.8
	12 Nite	76.1	74.7	75.0	75.6	62.2	63.1	62.2	63.5	69.1
	6 A.M.	75.7	76.4	76.1	74.4	62.0	66.0	64.4	63.4	69.9
VI	12 Noon	76.6	73.1	76.8	75.5	64.2	67.5	62.6	65.0	70.2
	6 P.M.	74.4	75.4	74.0	72.0	62.1	66.2	62.4	63.1	68.8
	12 Nite	73.9	75.5	75.8	75.3	63.3	64.3	63.8	63.0	69.4
	6 A.M.	75.8	75.0	74.3	75.3	63.7	63.8	62.1	64.6	69.4
VII	12 Noon	75.3	75.2	75.3	75.9	63.4	62.2	64.1	63.5	69.4
	6 P.M.	74.2	73.9	74.3	71.9	63.7	62.3	62.3	64.0	68.4
	12 Nite	76.6	75.8	76.9	73.7	64.0	62.4	62.2	63.6	68.9
Av. ± S.E.		75.4 ±0.18	75.1 ±0.20	75.6 ±0.15	75.1 ±0.40	64.6 ±0.38	65.6 ±0.41	64.1 ±0.35	65.2 ±0.29	70.1 ±0.18
	Av.	75.3				64.9				
Av.	6 A.M.	75.7	75.5	75.6	75.5	65.4	66.0	63.8	66.1	70.4
for	12 Noon	75.5	74.6	76.1	75.3	65.1	66.6	64.9	65.6	70.5
7	6 P.M.	74.7	75.2	74.9	74.2	63.7	65.0	63.8	64.7	69.5[b]
days	12 Nite	75.5	75.2	75.8	75.2	64.0	64.9	63.8	64.3	69.8
T.C.[a]		75.3	75.1	75.7	74.9	64.8	66.1	64.4	65.4	70.2
	Av.	75.2				65.2				

[a] Coefficients determined in conventional digestion trial.
[b] Highly significantly different from coefficients determined in conventional manner.

on the basis of these data, it is indicated that fecal samples obtained at 6 P.M. are not satisfactory for predicting dry-matter digestibility by the chromogen technique. The work of Bradley *et al. (1)* indicated that time of day is of little importance in obtaining fecal samples for the estimation of dry-matter digestibility by the plant-chromogen method.

Because the collection of feces extended over a period of seven days, and the forage used was advancing in stage of maturity, it is logical to assume that some decrease in digestibility would be observed from Day 1 to Day 7. Indeed, it was

shown by regression analysis that the bottom portion of the alfalfa decreased in digestibility about 0.6 of a percentage point per day. In contrast, no marked decrease in the digestibility of the top part of the forage was observed. There were probably several factors responsible for decrease in digestibility of the bottom half of the forage; however, the most logical one appears to be the decrease in the leaf-stem ratio that would be expected in this fraction as the trial advanced.

SUMMARY

Digestibility of the top and bottom portions of the alfalfa plant has been estimated from the concentration of plant chromogen in small samples of feces, collected at various times during a 24-hour period. The excretion pattern of this internal indicator was of the same general form as that reported by some workers for chromic oxide; however, the variation in excretion was considerably less than has been observed in most cases with chromic oxide. The excretion of plant chromogen was more uniform for animals receiving the top portion of the alfalfa plant than for animals receiving the bottom portion.

Dry-matter digestibility estimates determined by the plant-pigment method compared very closely with those determined by the conventional procedure (70.1 *vs.* 70.2%).

Estimates derived from fecal samples collected at 6 P.M. were significantly lower than dry-matter digestion coefficients determined by the total collection method. Dry-matter digestion coefficients predicted from feces voided at 6 A.M., 12 noon, and 12 midnight did not differ significantly from those determined by the conventional procedure.

Regression analysis revealed that the bottom portion of the alfalfa plant decreased in digestibility about 0.6 of a percentage point per day. On the other hand, no marked decrease in digestibility of the top part of the forage was observed as the trial advanced.

Under the conditions of this experiment, the bottom portion of the alfalfa plant was only 87% as digestible as the top portion.

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USE OF MECHANICAL REFRIGERATION IN PRESERVATION OF BULL SEMEN[1, 2, 3]

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Mechanical refrigeration was better than dry ice for maintaining semen quality for 24 weeks, as indicated by the percentage of living cells and their motility. Semen may be transferred back and forth from one storage to the other without decreasing its quality below that of dry-ice refrigeration. *Editor.*

Within the last few years, many of the procedures associated with the freezing of bovine semen have been refined, to make dissemination of the product more practical. In most of the early trials, dry ice was the refrigerant.

There is some indication at the present time that temperatures slightly warmer than dry ice are detrimental to spermatozoa. These observations have been reported by Miller and VanDemark *(4)* and Polge and Rowson *(6)*. Other workers *(1, 2, 3, 5, 7, 8)* also have shown that temperatures colder than dry ice are usually adequate for preserving semen quality, although some of the procedures used in obtaining low temperatures might be impractical. Since mechanical difficulties and operational expense often increase when substances are maintained at very low tempertures, it is important to determine the most practical means of storing frozen semen.

Beery and Ludwick *(1)* found that the quality (% alive and motility) of semen samples stored at a constant temperature of —96° C. in a mechanical freezer was superior to quality of those stored in a dry-ice unit.

The objective of the work reported here was to determine the quality of frozen semen stored under dry-ice refrigeration (approximately —74° C.) and mechanical refrigeration (approximately —96° C.), for various periods of time.

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[1] Contribution to North Central Regional Project NC-2, Improvement of Dairy Cattle Through Breeding.

[2] The material herein reported was taken from the data submitted by the senior author in partial fulfillment of the requirements for the degree, Master of Science, The Ohio State University, Columbus, Ohio, 1953.

[3] The study was made possible through the assistance of Central Ohio Artificial Breeding Association, Richard Kellogg, Manager.

EXPERIMENTAL PROCEDURE

Semen used in this study was obtained from bulls at The Ohio State University dairy herd and the Central Ohio Breeding Association. Twelve ejaculates from nine bulls were used.

In all cases, collections of semen were made by means of an artificial vagina. Immediately after collection, all semen was diluted at the rate of one part of semen to ten parts of warm (37° C.) egg yolk–citrate diluter. The diluter contained 3.6% sodium citrate, 25% egg yolk, and 500 units per ml. of Bicillin.

The semen was microscopically examined and evaluated for per cent live cells and comparative motility, immediately after collection and initial dilution. The motility score was based on a comparative rating of zero to nine. Fifty ml. of diluted semen were prepared for each ejaculate and cooled to 5° C. (±1° C.).

Final dilution was obtained by adding an equal volume of diluent containing 15% glycerol in five portions of five, seven, ten, 12, and 16 ml. at five- to eight-minute intervals. The glycerol diluter and all glassware used during glycerolization were cooled to refrigerator temperature (5° C. ±1° C.) prior to glycerolization. Addition of the glycerol diluter gave a final concentration of 7.5% glycerol by volume and 30 parts diluter to one part semen.

The glycerolated semen was allowed to equilibrate 12 to 18 hours at from 5° ± 1° C. After this period of time, each ejaculate was again microscopically examined to determine the effects of glycerolization. Part of each ejaculate was not glycerolated and was examined at this time for comparison.

After examination, one-ml. ampules were filled with the glycerolated semen. Eighty ampules of each ejaculate were filled and flame-sealed. After sealing, the ampules were placed in small trays and these trays were placed in an alcohol bath at 5° C. ±1° C., for the freezing process. Enough precooled alcohol was added to cover the semen in the ampules.

The temperature was lowered from +5° C. to 0° C. in about 15 minutes; then to −10° C. in about 20 minutes, and to −15° C. in five to six minutes, and, finally, to −75° C. in about five minutes.

Immediately after freezing, two ampules of each ejaculate were thawed in a water bath at 5° C. The semen in these two ampules was microscopically evaluated for per cent live cells and motility. A sample of each ejaculate of the unfrozen semen was also microscopically evaluated at this time.

Following the initial evaluation, 40 ampules of frozen semen from each ejaculate (in trays of alcohol) were placed under dry-ice refrigeration at −74° C. The other 40 ampules of each ejaculate were placed in a mechanical refrigerator at −96° C.

One week after the ampules of semen had been placed under the two storage conditions, two ampules of each ejaculate in each group were thawed in a water bath of 5° C., and microscopically evaluated by two men, working independently.

After three and 12 weeks of storage under the two conditions, a portion of each ejaculate was transferred to the opposite storage condition. Two ampules from each ejaculate under each of the transfer conditions were thawed and evaluated at one, three, nine, and 21 weeks after transfer.

Average ratings of the 12 ejaculates at each evaluation, under each storage and transfer condition, were calculated. The probability of observed differences occurring through chance alone, if no actual differences existed between the treatments, was determined by use of the "t"-test for paired comparisons, as outlined by Snedecor *(9)*.

RESULTS AND DISCUSSION

Average evaluations of semen quality, as indicated by per cent of live cells and motility, are presented (Table 1). There is some indication from these

TABLE 1

Means of evaluations for per cent alive and motility of sperm of 12 split-samples, stored continuously at two low temperatures

Time of evaluation after freezing	Mechanical (−96° C.)		Dry ice (−72.6° C.)	
	% Alive	Motility	% Alive	Motility
1 week	37.5[b]	6.42	29.2	6.50
3 weeks	45.4[b]	6.50	31.7	5.67
4 weeks	41.7[a]	6.58	37.5	5.83
6 weeks	41.7	6.58	36.7	6.42
12 weeks	42.9[a]	7.17[a]	37.1	6.25
13 weeks	45.4[b]	6.75	38.8	6.33
15 weeks	45.4[b]	7.08[b]	33.8	6.17
24 weeks	46.2[b]	6.83	33.3	6.50
Average of all readings	43.3[b]	6.74[b]	34.8	6.21

[a] Significant at 5% level.
[b] Significant at 1% level.

data that the quality of semen stored in the mechanical freezer was somewhat better than that stored under dry-ice refrigeration. The consistent difference between the samples is not great, although statistical tests indicate a good possibility of true differences.

The slight increase in average evaluation with increased storage time is somewhat difficult to explain. This apparent increase in quality after several weeks of storage has been observed previously *(1, 6)*. Several opinions can be proposed which might partially explain this occurrence. *(a)* There is a possibility that some adjustment period is needed, for sperm to respond to the freezing and thawing processes. This period of adjustment has been assumed to be approximately three weeks *(1)*. *(b)* There may be some disintegration of sperm during the first few weeks of storage. If these cells can not be observed following the thawing process, the ratio of live to dead cells may appear to change. *(c)* Personal bias may be a factor in all types of semen evaluation. As the samples become older, evaluators may tend to compensate for this by increasing the estimates slightly.

In Table 2, comparisons of evaluations are made between the two storage conditions. If mechanical refrigeration is more practical than dry ice for frozen semen storage, some semen-processing establishments may want to move the samples from a central area, which uses mechanical refrigeration, to the

TABLE 2

Comparison of means of evaluations for per cent alive and motility of sperm of 12 frozen samples, transferred at various intervals from two low temperatures

Comparison	Interval after freezing: 4 wk.		6 wk.		12 wk.		13 wk.		15 wk.		24 wk.		Av. of all readings	
	%A	*Mot.*	*%A*	*Mot.*	*%A*	*Mot.*	*%A*	*Mot.*	*%A*	*Mot.*	*%A*	*Mot.*	*%A*	*Mot.*
1 (a)[c]	41.7	6.58	41.7	6.58	42.9	7.17	45.4	6.75	45.4	7.08	46.2	6.83	43.9	6.83
(b)[d]	36.7	5.92[a]	32.5[a]	6.17[b]	36.7[a]	6.58[a]	33.8[b]	6.25	35.0[b]	6.42[a]	35.4[b]	6.53[b]	35.0[b]	6.28[b]
(e)[e]	32.1[b]	5.75[b]	33.3[a]	5.92[a]	28.8[b]	6.33[b]	36.7[a]	6.58	35.8[b]	6.33[a]	35.8[b]	6.50[a]	33.8[b]	6.24[b]
2 (a)[f]							36.7	6.25	35.4	6.42	35.8[a]	6.33	36.0	6.33
(b)[g]							37.1	6.00	35.8	6.50	39.1	6.33	37.3	6.28

[a] Significant at 5% level; [b] significant at 1% level; [c] continuous mechanical refrigeration; [d] transferred from mechanical to dry ice after three weeks; [e] transferred from dry ice to mechanical after three weeks; [f] transferred from mechanical to dry ice after 12 weeks; [g] transferred from dry ice to mechanical after 12 weeks. In comparison 1, d and e are compared with c, and in comparison 2, g is compared with f..

technician's area, where dry ice is used as a refrigerant. Because of the practicability of this procedure, transfers of semen from each of the storage conditions were made at various periods.

In general, it is observed that samples retain a higher quality, as indicated by per cent alive and motility of sperm, when stored continuously under mechanical refrigeration. When samples are moved from one storage condition to the other, their quality tends to be reduced slightly, but not below the quality associated with the warmer storage condition (dry ice).

Although definite reasons for the difference in quality of semen stored under the two conditions are not known, these two may be likely: *(1)* It may be that dry-ice refrigeration is very near the critical temperature for semen storage. When dry ice is used as a refrigerant, it is often difficult to maintain all of the stored products at a constant temperature. The variations would be dependent, to a large degree, on the size and type of storage box used and the quantity of dry ice maintained. *(2)* In addition to the differences in actual temperatures of the two storage conditions, the mechanical unit maintained a a more even temperature than the dry-ice box. Temperature variations of 4 to 6° C. were noted in the dry-ice unit. These changes usually occurred when fresh supplies of dry ice were added to the box. The recording thermometer on the mechanical box indicated only small (one- to two-degree) changes. Thus, the actual difference, or fluctuations, in temperatures could have influenced the semen quality.

SUMMARY

Twelve ejaculates from nine bulls were frozen and stored under mechanical and dry-ice refrigeration. Portions of each ejaculate remained under the two storage conditions for a 24-week period. At various intervals, semen samples were transferred from one storage condition to the other. Samples were evaluated at the end of each period for per cent of live cells and motility, to obtain an estimate of the quality of the sample.

Under conditions of the experiment, evaluations of the samples indicate that mechanical refrigeration is superior to dry-ice refrigeration in maintaining quality of frozen semen. It is also indicated that semen samples may be transferred from mechanical refrigeration (−96° C.) to dry ice, and vice versa, without reducing their quality below that normally obtained by dry-ice refrigeration.

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IN VITRO STUDIES ON METHANOGENIC RUMEN BACTERIA

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Rumen cultures produced methane, from formic and acetic acid at 15° to 45° C., but they fermented butyric acid at 45° C. Those fermenting acetate were inhibited by carbon dioxide, dyes, and penicillin, but the formate fermenters were unaffected. A rumen organism isolated from formate medium was tentatively identified as *Methanobacterium formicicum* **and one from the acetate medium resembled** *Methanobacterium sohngenii*, **but differed in cell arrangement.** *Editor.*

Large quantities of methane are produced in the forestomach of the ruminant, but few data are available on rumen methane-producing bacteria. Methane bacteria from mud and sewage have been studied extensively (*5*, *12*). Some of the substrates utilized by bacteria from the latter sources for methane production are also the products of carbohydrate dissimilation in the rumen. They include volatile fatty acids (*14*) and hydrogen and carbon dioxide (*10*). Low molecular weight alcohols (*2*) and carbon monoxide (*7*) also serve as substrates. Some higher fatty acids, carbohydrates, and other compounds such as succinic acid (*5*) are utilized for methane production by impure sewage cultures. Carbohydrates do not serve as substrates for those methane bacteria which have been studied in pure culture (*2*, *13*, *16*). In addition to the substrate limitations of the methane bacteria as a group, the individual species exhibit a rather high degree of substrate specificity. Indeed, some species characteristically may be restricted to the utilization of one or two compounds (*15*).

Conversion of acetic acid to methane and carbon dioxide by a Methanosarcina species isolated from goat rumen fluid has been reported (*3*). The relative rates at which methane was produced from succinate and formate by rumen fluid from the same source suggested that formate is the primary substrate for methane formation in the rumen. Washed-cell suspensions of mixed rumen bacteria incubated under nitrogen mediated methane formation from formate, maltose, and pyruvate (*9*). Glucose, cellobiose, and possibly lactate were utilized for methane production when the mixed washed cells were incubated under hydrogen.

Although the methanogenic capacities of rumen fluid and of washed cell suspensions of mixed rumen bacteria have been described, knowledge is limited concerning the types of rumen bacteria responsible for the formation of methane, and their activities in culture. Accordingly, experiments were designed to

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describe the biochemical activities, cultural characteristics, morphology, and probable identity of these organisms. For this purpose, enrichment cultures were established and stabilized as a prelude to attempts to isolate rumen methane bacteria. Observations concerning morphological types, environmental conditions favoring their development, and substrate preferences also were made by use of the stabilized enrichment cultures.

METHODS AND MATERIALS

Bacteriological. The general type of fermentation apparatus described by Buswell *et al.* (*6*) was used in this study of the anaerobic conversion of fatty acids to methane by stabilized enrichment cultures from bovine rumen fluid.

The fermentation mixture contained 125 ml. Salt Solution A and 7 ml. Salt Solution B (*1*), three gm. acid-washed asbestos fibers as nidus (*4*), and 125 ml. of cheese cloth–filtered fresh rumen fluid as inoculum. The pH of the fermentation mixture was 6.8. Salt Solution A was prepared by dissolving 0.01 gm. $MgCl_2$, 0.05 gm. K_2HPO_4, and 0.01 gm. NH_4Cl in 500 ml. of distilled water. Salt Solution B contained 5.0 gm. Na_2CO_3 and 1.0 gm. $Na_2S{\cdot}9H_2O$ dissolved in 100 ml. distilled water.

Anaerobiosis was established by flushing the apparatus with a stream of methane, introduced through a hypodermic needle inserted through the rubber serum cap of the reaction flask. Salt Solution B and substrate were introduced in the same fashion. The substrate used was either 0.9 *N* formic, 0.65 *N* acetic, 0.5 *N* propionic, or 0.32 *N* butyric acid. Substrate was added to the cultures daily in the amount of from 1.0 to 4.0 ml., depending upon the rate of gas production.

The cultures were incubated at 37° C. for several weeks and observed daily for changes in composition of the microflora, as indicated by Gram stain and cell morphology. Measurements of cultural characteristics, including rate of substrate utilization, rate of gas formation, gas composition, and effects of various inhibitors were made on stabilized cultures. Procedures employed by Barker (*2*) and Mylroie and Hungate (*10*) were used in attempts to obtain pure cultures of methanogenic species from these cultures.

Chemical. Gas from cultures was collected by the displacement of acidified (pH 1.0) saturated aqueous Na_2SO_4. The volume of gas produced was recorded daily, and gas composition was measured by absorption-combustion procedures, employing a Model No. 35-802 Burrell apparatus.

Total volatile acidity was measured by steam distillation of acidified 1.0-ml. aliquots of the growth medium in a Markham apparatus (*8*). Distillates were titrated with 0.01 *N* aqueous NaOH to a methyl red end-point in a stream of CO_2-free air.

Concentrations of the individual fatty acids in the fermentation mixture were measured by chromatography, employing a 300×12 mm. column packed with 6 gm. Celite No. 535 which had been acidified with 2.4 ml. of 0.2 *N* H_2SO_4 and slurried in hexane equilibrated against 0.2 *N* H_2SO_4. The acids from one ml. of acidified fermentation mixture packed on the column in 2 gm. of Celite

were eluted by butanol-hexane solvents *(11)*. The eluate was collected in 5-ml. fractions and titrated to a bromthymol blue end-point with 0.01 *N* alcoholic KOH in a stream of CO_2-free air.

RESULTS

Stabilized rumen cultures which degraded acetic and formic acids to methane and carbon dioxide were readily established at 37° C. Butyric acid–stabilized cultures were obtained at 45 but not at 37° C.; however, cultures established at 45° could be maintained in an active state at 37° C.

Numerous attempts to stabilize cultures using propionic acid as substrate were unsuccessful. In addition, the propionic acid contributed to the fermentation mixture by the rumen fluid inoculum was not utilized in cultures actively producing methane from formic and acetic acids.

Since the methane bacteria characteristically develop slowly, a period of two weeks generally elapsed from the time the cultures were set up until gas evolution became consistently rapid. Gas produced during the first few days of the stabilization period contained methane as the major component. By the end of four weeks, the volume and composition of the gas approximated theoretical values *(18)* calculated from the amount of added substrate utilized, and the cultures were completely dependent upon the daily addition of substrate. Analysis of the fermentation mixture at this stage showed that only the propionic fraction of the volatile acidity of the rumen fluid remained. Data shown (Table 1) illustrate the changes in gas composition and in volatile acidity during the stabilization period in which both endogenous and added substrates were being fermented. Theoretical gas volumes were calculated on the basis of added substrate.

The major changes in flora of acetic acid and formic acid fermentation mixtures occurred during the first seven to ten days of incubation, and appeared to be independent of the type of substrate added. A Gram-negative micrococcus predominated all stabilized cultures. Minor changes in flora were substrate-dependent and the associated organisms present in considerable numbers included a Gram-positive macrococcus, a short, thick, Gram-variable rod, and a long, slender, Gram-negative rod. The latter type especially was encountered in cultures stabilized to formic acid. The short, Gram-variable rod forms were found primarily in cultures metabolizing acetic acid.

Changes in the flora were detected when cultures stabilized to acetic acid were given formic acid as the sole substrate, and vice versa. A few days after acetate cultures were fed formic acid, slender, Gram-negative rods similar to those in the formate cultures appeared in greater numbers. When formic acid cultures were fed acetic acid, the short, thick Gram-variable rods gradually increased in number. Concurrent with the substrate-induced changes in flora, the CO_2:CH_4 ratio of the gas gradually changed toward the theoretical ratio calculated for the new substrate. Changes in composition of the gas are shown (Table 2). After 20 days on the alternate substrate, the cultures readily utilized the original substrate, and gas composition returned to that characteristic of

TABLE 1

Changes in volatile acidity and gas composition during culture stabilization[a]

Incubation	Acetate cultures Gas/24 hr.					Formate cultures Gas/24 hr.				
	Volatile acidity	Volume, cc.		Composition		Volatile acidity	Volume, cc.		Composition	
(days)	*(meq/ml)*	*(actual)*	*(theory)*	*(% CO_2)*	*(% CH_4)*	*(meq/ml)*	*(actual)*	*(theory)*	*(% CO_2)*	*(% CH_4)*
16	0.064	40	74	15	88	0.054	55	50	18	48
21	0.061	94	74	42	57	0.043	80	75	54	45
27	0.035	94	74	49	51	0.029	85	75	58	42
40	0.025	85	111	50	49	0.006	70	75	67	32
42	0.019	98	111	46	53	0.005	70	75	69	29

[a] After onset of gas production (12 days), substrate additions were made in accordance with amount of gas produced per 24 hr. Acetate was fed at rate of 0.65 m*M*/37 cc. of gas, and formate at rate of 0.9 m*M*/25 cc. of gas until endogenous substrate was depleted. Data represent $\bar{x}$ values from three cultures for each substrate.

TABLE 2

Substrate-induced changes in composition of gas produced by stabilized cultures[a]

Time after substrate change	Acetate stabilized cultures changed to formate						Formate stabilized cultures changed to acetate					
	Gas/24 hr.			Volatile acidity			Gas/24 hr.			Volatile acidity		
	Volume	Composition		Total	Formic	Acetic	Volume	Composition		Total	Formic	Acetic
(days)	*(cc.)*	*(% CO_2)*	*(% CH_4)*	*(meq/ml)*	*(meq/ml)*	*(meq/ml)*	*(cc.)*	*(% CO_2)*	*(% CH_4)*	*(meq/ml)*	*(meq/ml)*	*(meq/ml)*
0	108	49	44	0.023	0.000	0.018	70	72	30	0.005	0.007	0.000
1	70	56	43	0.038	.....	.....	90	75	23	0.016	.....	.....
2	61	58	41	0.042	0.031	0.007	40	64	35	0.032	0.000	0.017
3	47	....	....	.....	.....	.....	23	....	....	.....	.....	.....
4	28	66	38	0.048	.....	.....	52	49	50	0.040	.....	.....
5	56	61	38	0.040	0.028	0.000	52	50	48	0.045	0.000	0.033
6	75	62	37	0.038	.....	.....	58	49	50	0.041	.....	.....
7	60	65	33	0.030	.....	.....	80	50	49	0.037	.....	.....
8	75	68	32	0.025	.....	.....	....	....	....	.....	.....	.....
9	65	69	30	0.021	.....	.....	....	....	....	.....	.....	.....
10	....	....	....	.....	0.008	0.000	....	....	....	.....	0.000	0.017

[a] After substrate was changed, alternate substrate was added in accordance with gas production (Table 1). The data represent $\bar{x}$ values from duplicate cultures.

the original substrate. The acetic acid cultures did not utilize acetate after being fed formic acid for five weeks. Formic acid cultures fed acetate for five weeks were able to utilize the original substrate.

Since it was evident that rumen fluid contained organisms which converted both acetic and formic acid to methane, an experiment was designed to test the hypothesis that these substrates are utilized simultaneously by a mixed rumen flora. Accordingly, new cultures were stabilized to a multiple substrate consisting of both formic and acetic acid. When stabilized, the cultures yielded theoretical volumes of gas for the amount of substrate degraded. However, the data presented (Table 3) demonstrate a preferential utilization of formate over acetate. The formic acid disappeared rapidly from the medium, and when the

TABLE 3

Preferential utilization of formic acid by culture stabilized on both formic and acetic acid [a]

Incubation after substrate added	Substrate concentration		Gas volume/hr.
	Acetic Acid	Formic Acid	
(*hr.*)	(*μM/ml*)	(*μM/ml*)	(*cc.*)
2	15.6	13.8	22
3	14.5	12.1	21
4	15.8	11.0	10
6	14.5	6.0	19
10	14.5	0.0	24
20	9.5	0.0	38
28	9.2	0.0	12

[a] 1.8 m*M* of formic acid and 1.3 m*M* of acetic acid were added at zero-time; 0.9 m*M* of formic acid was added after one hour.

formic acid level was maintained above exhaustion, little or no acetate was fermented. After the medium was depleted of formic acid, acetic acid was used for methane production at a rate comparable to that of cultures stabilized on acetate only.

The delayed utilization of acetate in the presence of formate may have been accentuated by the large amounts of carbon dioxide produced by the fermentation of formic acid. Observations made earlier in this study indicated that the activity of newly started acetate cultures was delayed for 20 days when they were initially swept with carbon dioxide. In contrast, acetate cultures swept with either nitrogen, hydrogen, or methane become active after nine to 11 days. Formic acid cultures were not affected by the type of gas used to establish anaerobiosis. The passage of 300 cc. of carbon dioxide through acetate-stabilized cultures reduced gas formation from control values of 90 cc. of gas per eight hr. to 40 cc. per eight hr. for the treated cultures. The same volume of carbon dioxide did not affect the rate of gas formation from formic acid. Continuous passage of carbon dioxide through stabilized cultures for 24 hr. reduced acetate utilization approximately 40 per cent. Formate utilization under these circumstances was not altered.

Cultures stabilized to acetate or formate had a relatively wide growth temperature range, 15 to 45° C. Formate cultures producing 90 cc. of gas per 24 hr. at 37° C., produced 97 cc., 81 cc., and 50 cc. of gas per 24 hr. when incubated

at 45, 26, and 15° C., respectively. Acetate cultures producing 95 cc. of gas per 24 hr. at 37° C., produced 118 cc., 87 cc., and 55 cc. of gas when incubated at 45, 26, and 15° C., respectively. Both types of cultures failed to produce gas at 55° C.

Prior to attempts to isolate methane bacteria from cultures stabilized to formic or acetic acid, an effort was made to restrict the flora by the addition of crystal violet, brilliant green, penicillin, sulfanilamide, or sodium desoxycholate. The addition of the inhibitors caused only negligible changes in the flora. However, gas production ceased two days after the addition of brilliant green (52 μg/ml), penicillin (100 units/ml), or sodium desoxycholate (130 μg/ml) to acetate cultures. The effect of crystal violet (5 μ/ml) was less pronounced, in that 14 days were required for complete loss of activity. The activity of formate cultures was not affected by the same concentration of brilliant green, penicillin, or crystal violet. The addition of sodium desoxycholate to formate cultures caused a gradual reduction in daily gas volume until no gas was formed after seven days. Neither type of culture was affected by one mg/ml sulfanilamide.

The procedures employed for the isolation of methane bacteria from the stabilized formate cultures were those described by Mylroie and Hungate (*10*). Using hydrogen and carbon dioxide as substrates, three sequential transfers of isolated colonies were made in agar. The colony and cell morphology of the isolates which appeared after eight days of incubation closely resembled that of *Methanobacterium formicicum*. These isolates failed to grow when replaced in liquid medium.

Attempts were made also to partially purify the flora of stabilized formic acid cultures by a series of transfers in liquid medium. When 25 ml. of active culture was transferred to a fermenter containing only Barker's medium (*1*), growth was not established. However, when Barker's medium was supplemented with an equal volume of sterile rumen fluid, the culture survived and rapid gas production occurred within four days after transfer. After 22 days of stabilization, this culture consisted primarily of the long, slender Gram-negative rods previously seen in formate cultures, and having the morphology of *Mb. formicicum*. The gas volume and ratio of CO_2:CH_4 produced by this culture from formic acid was in accord with the predicted theoretical values (*18*). The culture was carried through two additional transfers in the same manner. The final transfer resulted in a highly purified methane-producing culture containing very few contaminating forms. The morphology of the predominate form was identical to that of the organisms from colonies grown anaerobically in the presence of carbon dioxide, with hydrogen as the sole source of energy. The cell and colony morphology, and the biochemical characteristics of this culture, suggest its probable identity as *Mb. formicicum*.

Attempts to isolate pure cultures of the methane bacteria from stabilized acetic acid cultures were made, using Barker's procedure (*9*). The short, plump, Gram-variable rod seen in acetic enrichment cultures was the only form that grew in this acetate-mineral medium. Colonies of this organism were carried

through four successive shake-culture transfers in agar, and finally placed in liquid medium enriched with one-half sterilized rumen fluid. After a lag period of two weeks, the organism grew for a period of nine days and produced a total of 135 cc. of gas, which contained 40% methane.

DISCUSSION

The foregoing data indicate that rumen fluid contains bacteria capable of converting formic, acetic, and butyric acid to methane and carbon dioxide. Stabilized enrichment cultures which metabolized these substrates were obtained. Attempts to establish stabilized methane cultures on propionic acid were unsuccessful. The latter finding is consistent with the observations of other investigators *(9)* and suggests that propionic acid is not a substrate for the formation of methane in the rumen.

The experimental data indicate that different rumen organisms are responsible for the fermentation of formic and acetic acid. The morphological, biochemical, and cultural characteristics of the formate-fermenting rumen organism closely parallel those described for *Mb. formicicum* (*10*, *13*). The data suggest a tentative identification of the rumen species as *Mb. formicicum*, and that this organism is a normal inhabitant of the rumen. The tentative identification is also supported by the consideration that *Mb. formicicum* is the only Gram-negative, rod-shaped bacteria described in the literature as capable of producing methane from formic acid.

The probable identity of the acetate-fermenting rumen organism is more obscure. According to the literature, *Methanobacterium sohngenii* is the only rod-shaped methane bacteria capable of fermenting acetic acid (*14*). However, the acetate-fermenting organism observed in stabilized rumen cultures, and carried through isolation and purification procedures, appeared as a short, plump Gram-variable rod, and not in the long-chain arrangement characteristic of *Mb. sohngenii* (*14*). The rumen organism, therefore, may be a morphologically atypical *Mb. sohngenii* affected by some cultural conditions which prevent chain formation, or the organism may be a heretofore-undescribed species inhabiting the rumen.

Data relative to the preferential utilization of formate in the presence of acetate substantiate other reports (*3*, *9*) which suggest that formic acid is a primary substrate for methane formation in the rumen. Since the formate-utilizing cultures are capable of producing methane from the reduction of carbon dioxide by hydrogen, these gases also may serve as a source of rumen methane. The reduction of carbon dioxide, coupled with the oxidation of butyric or other fatty acids, is an additional possibility for rumen methane formation. The relatively low rate of activity of butyrate-stabilized cultures argues against the importance of butyric acid as a main source of rumen methane.

The high degree of substrate specificity of known methane bacteria, coupled with the presence of acetate-utilizing methane organisms in the rumen fluid, suggests that some rumen methane may be derived from acetic acid. The exact proportion of rumen methane arising from the formate–carbon dioxide pathway

vs. the acetate pathway is unknown. Indirect evidence does suggest that the former system is of major importance. Definition of the relative importance of these pathways is critical to attempts to increase feed utilization efficiencies by reducing methane production. If a considerable portion of rumen methane arises from acetate, then prevention or reduction of methane formation would be of direct nutritional value. If the major portion of rumen methane arises from the reduction of carbon dioxide, blocking the conversion of carbon dioxide to methane should be of some value from the standpoint of conservation of total energy in the rumen. However, it is not known to what extent the host would benefit from this conservation.

If the conversion of formic acid proceeds via hydrogenlyase to CO_2 and H_2, and these products via hydrogenase to methane, as has been postulated (*17*), blocking methane formation at the CO_2 reduction stage again should be of some value, as noted previously. The value of a metabolic block which would result in the accumulation of formic acid in the rumen is unknown, because the nutritional significance of formic acid in ruminant metabolism has not been defined.

Thus, it is apparent that data from critical in vivo experiments designed to define the major pathways of methane formation in the rumen are essential to a realistic evaluation of the physiological importance of rumen methanogenesis.

SUMMARY

Stabilized cultures which produced methane from formic and acetic acids were obtained from rumen fluid. Cultures utilizing butyric acid could be established only at 45° C. All attempts to stabilize cultures on propionic acid were unsuccessful.

Rumen cultures stabilized to formic or acetic acid produced methane at 15 to 45° C. Cultures stabilized to both formic and acetic acid preferentially and completely utilized the formic acid before acetic acid was fermented.

Acetate-fermenting cultures were inhibited by carbon dioxide, dyes, and penicillin; whereas, formate cultures were unaffected by these agents. The methane organism obtained in a highly purified state from formate-stabilized rumen culture closely resembled *Mb. formicicum* and was tentatively identified as such. The methane bacteria obtained from rumen cultures stabilized on acetate, physiologically resembled *Mb. sohngenii*, but differed in the morphological arrangement of the individual cells.

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SOME EXPERIMENTS IN PRESERVATION OF HIGH-MOISTURE HAY-CROP SILAGES

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Experiments were conducted to determine the effects of adding cornmeal, beet pulp, and Kylage, and the recirculation of seepage, to improve the quality of hay-crop silage. Cornmeal and beet pulp improved nutrient preservation and feeding value of the silage, but forage dry matter consumption was less as compared to the controls. Kylage did not improve the silage. These additives may be expected to improve poor-quality but not good-quality silages. Recirculation of seepage was impractical because there was only slight improvement of the silage and the necessary equipment was costly. *Editor.*

Reports on the use of preservatives, to improve the efficiency of nutrient preservation, chemical quality, and feeding value of silage made from high-moisture hay crops, have been numerous. Lack of agreement as to the necessity of, or expected benefit from, these preservatives is quite apparent. Allred *et al. (1)* and Wittwer *et al. (9)* reported that neither sodium meta-bisulfite, molasses, nor Brewer's dried grains decreased storage losses or improved feeding value sufficiently to warrant their use.

Bratzler *et al. (5)* reported marked improvement in nutrient preservation from the use of sodium meta-bisulfite. Improvement of chemical quality through the use of Kylage [2] or sodium meta-bisulfite was observed by Archibald *(2)*. Variability between control silages was observed by Gordon *et al. (6)* to be the primary factor influencing the apparent benefit obtained from preservatives. Bohstedt *et al. (4)* reported good preservation of alfalfa silage by 150 to 200 pounds of added cornmeal. King and Bender *(7)* concluded that if no other source of concentrate was provided for high-producing cows, more than 500 lb. of cornmeal per ton of alfalfa would be required in the silage-concentrate mixture, to provide sufficient nutrients.

The extent of dry matter losses through seepage in untreated silage has been reported as ranging from less than 1.0% to about 8.0% *(1, 3)*. Such

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[2] A patented preservative composed mainly of sodium nitrite and calcium formate in dry powder form.

factors as moisture content of the original crop, silo size, accuracy of seepage collection, and extent of leaching from rainwater may contribute to differences in observations. Thus, the potential benefit of seepage absorption by feedstuff additives is also variable.

The two experiments reported herein were conducted to determine the relative effectiveness and efficiency of adding cornmeal, beet pulp, and Kylage, and recirculation of seepage, as methods of improving the chemical quality, preservation efficiency, and feeding value of high-moisture hay-crop silage.

EXPERIMENTAL PROCEDURE

The crops used in this investigation were harvested in 1954 (Experiment 1) and 1955 (Experiment 2) and fed during the winters following each harvest.

Experiment 1. A first-cutting mixture of orchard grass and Ladino clover (less than one-fourth clover) was harvested without wilting, with a windrow pick-up forage harvester set for a ¾-inch cut. Most of the orchard-grass heads had emerged but were not in bloom; very few clover blooms were present.

Alternate loads of the chopped forage were stored in three 10-foot × 25-foot concrete stave silos. Cornmeal at the rate of 200 lb. per ton of forage was spread on top of the loads of forage before being blown into the first silo. Kylage at the rate of 4.3 lb. per ton was added at the blower to forage of the second silo. A control forage to which nothing was added was stored in the third silo. Each load of forage was weighed and sampled. The average composition of the cornmeal-treated silage as stored was calculated from separate weights, samples, and analysis of forage and cornmeal, as recommended *(8)*.

Initial filling of the silos was done from May 11 to May 14. Temporary covers of plastic, weighted with nonexperimental forage, were then applied. On May 18 the covers were removed and the silos refilled. All silos were capped with plastic, weighted with a load of fresh, untreated forage.

Seepage was collected in gutters surrounding the silos and measured with steam-distillate meters during the period of high flow rates, and by collection cans during the remainder of the storage period. Aliquot samples of the seepage were collected for dry matter determination. Temperatures at several points in the silages were measured with buried thermocouples and recorded automatically throughout the storage period.

After a storage period of about five months, the silos were opened and the silage removed during the next four months. Top spoilage was separated and removed and analyzed for dry matter. All feedable silage was weighed as removed from the silo. Samples taken each five days were analyzed for pH, dry matter, carotene, sugar, ammoniacal nitrogen, and acetic, propionic, butyric, and lactic acids. A portion of each sample was reserved under refrigeration and compounded by 20-day periods for proximate analysis. Analytical methods were those recommended at a conference of silage investigators *(8)*.

Feeding value of the silages was evaluated with 12 milking cows in a 3 × 3 Latin-square feeding trial. The trial consisted of three 40-day periods (ten days change-over and 30 days experimental), or a total of 120 days.

The ration consisted of the experimental silage, fed to the extent of appetite, and grain fed according to milk production (one grain: four FCM). The grain ration was adjusted according to individual production at the beginning and end of each preliminary period, on the basis of production during the previous ten days. Thereafter, adjustments were made each ten days, based on the average decline in production of all the cows. The grain ration was composed of: cornmeal, six parts; ground oats, four parts, and one per cent salt and one per cent steamed bonemeal. A special adjustment in the grain ration was made, in order to observe to what extent the cornmeal used as a preservative was still available as a supplement at the time of feeding. It was assumed that ten per cent by weight of the treated silage was cornmeal (the approximate ratio at the time of storage); therefore, the treated silage ration was supplemented with grain only when ten per cent of the silage weight consumed was less than the grain required by the 1:4 grain:milk ratio.

Data were obtained on a daily individual-cow basis for the weights of feeds fed and refused and milk produced. Percentage butter fat in the milk was determined for each cow from two-day composite samples taken once during each ten-day period. The dry matter content of refused feed was determined at five-day intervals and that of grain fed, each 20 days. Sampling and analysis of silages fed have been previously described.

Experiment 2. The methods and procedures of Experiment 2 were the same as for Experiment 1, with exceptions as indicated. The crop harvested April 28 to May 2, although of the same type, was somewhat less mature, with only about 20% of the orchard-grass heads emerged at harvest. The treatments used were *(1)* 200 lb. of dried beet pulp per ton of green forage ensiled; *(2)* no additive to the green ensiled forage, but recirculation of seepage, and *(3)* control, or no treatment.

The beet pulp treatment was carried out in the same manner as the cornmeal treatment of Experiment 1. Recirculation of the seepage for the 11-day period, April 29 to May 10, was accomplished by collecting the sepage in a gutter surrounding the silo and draining into a collection pit. For the silo in which the seepage was recirculated, a pump activated by a float switch pumped the seepage to the top and applied it to the top surface of the silage, through a systematically arranged garden-type soaker hose. Run-off of the seepage was allowed following the recirculation period. Seepage losses from all silos were measured by the system described under Experiment 1.

The silos were filled during the period from April 28 to May 2 and covered with commercial plastic silo caps, the seepage return pipe being inserted through the silo cap. No refilling was done, because of interference with the recirculation system.

The silos were opened after three months of storage and contents fed during the next four months. The silage sampling procedure in this experiment consisted of compounding daily samples by five-day periods for the first 20 days and by ten-day periods thereafter. The composite samples were used for all analyses.

TABLE 1

Chemical composition of materials stored

Treatment	Dry matter	Composition of dry matter						
		Protein	Ether extract	Fiber	N-free extract	Ash	Carotene	Sugar
	(%)						(μ/gm)	(%)
Experiment 1								
Control[a]	18.4	16.7	2.7	22.8	48.0	9.8	244	8.8
Kylage[a]	18.1	17.0	2.7	23.7	46.7	9.9	237	9.2
Kylage[b]	18.3	16.8	2.7	23.4	46.2	10.9	234	9.1
Cornmeal[a]	17.8	17.0	2.8	23.3	46.8	10.1	247	9.3
Cornmeal[b]	24.4	14.5	3.2	16.1	58.4	7.8	164	6.7
Experiment 2								
Control[a]	18.1	19.4	2.7	21.1	46.6	10.2	297	7.1
Recirculated[a]	16.8	20.1	3.0	21.6	44.5	10.8	305	7.6
Beet pulp[a]	17.4	20.0	2.9	21.0	45.5	10.6	315	7.0
Beet pulp[b]	23.7	16.6	2.1	21.0	52.2	8.1	212	6.8

[a] Composition of forage only.
[b] Calculated composition of mixture from separate analysis of components.

The feeding trial design and procedure was the same as in Experiment 1. The grain ration for cows fed beet pulp treated silage was reduced by ten per cent of the weight of the silage consumed. This reduction was sufficient to result in no grain supplement being fed to these cows.

RESULTS

The chemical composition of the materials ensiled is presented (Table 1). The values presented are weighted averages of all the values obtained. The higher protein and carotene, and the somewhat lower crude fiber content of the 1955 forage, appear to be related to the earlier stage of maturity, as compared to the 1954 forage. Percentage dry matter was quite similar in the forage portion of all silages.

Silage temperatures near the center of the mass in the cornmeal and control silages of Experiment 1 changed very little during the entire storage period. Temperatures at filling of about 78° F. gradually increased to a peak of 84° F. in mid-August, about 100 days after filling, then declined with the onset of lower ambient temperatures. Temperatures in the Kylage-treated silage increased from 63 to 73° F. during the first two weeks. The more gradual rise that followed, peaked at about 86° F. in mid-August, about 115 days after filling. A slow decline followed this peak.

Temperatures near the center of the mass in all of the Experiment 2 silages seldom differed by more than 5° F. between treatments. Aside from three unexplained deviations, which were thought to be technical errors, temperature changes can best be described as a very gradual rise from an initial temperature of about 76° F. at the time of ensiling, to about 83° F. by the first of September, followed by a gradual decline.

The average chemical composition of the silages as removed is presented (Table 2). All silages increased in percentage dry matter. This was most

TABLE 2

Chemical composition of silages

Treatment	Dry matter	Composition of dry matter											
		pH	Protein	Ether extract	Fiber	N-free extract	Ash	Carotene	Sugar	Ammoniacal[a] nitrogen	Butyric Acid	Lactic Acid	Acetic Acid	Propionic Acid
			(%)					(μ/gm)		(%)				
Experiment 1														
Control	24.0	3.76	16.0	4.6	28.4	43.2	7.8	281	0.5	1.169	Trace	7.8	2.0	0.1
Kylage	24.6	3.67	16.0	4.3	27.9	43.5	8.3	265	0.8	0.981	Trace	8.0	1.5	0.1
Cornmeal	30.6	3.72	13.8	4.7	20.2	55.1	6.2	182	0.5	1.034	0.1	6.6	1.4	0.1
Experiment 2														
Control	22.1	4.44	19.3	5.1	26.9	38.0	10.7	339	0.2	2.886	1.1	4.7	4.2	0.7
Recirculated	22.3	4.39	19.0	5.7	26.2	38.7	10.4	332	0.2	2.458	0.4	5.2	4.2	0.5
Beet pulp	25.6	3.90	17.0	3.7	23.7	46.4	9.2	237	0.9	1.532	0.3	6.7	4.6	0.4

[a] Expressed as protein.

marked in the silages of Experiment 1 and least in the beet pulp treated silage. The silages of both experiments were characterized by typical changes in the percentages of crude constituents in the dry matter. Increases in ether extract and crude fiber, and decreased nitrogen-free extract, were the most marked and consistent. Increases in concentration of carotene and decreases in levels of sugar (which had been largely utilized in the fermentation) were common to all the silages.

The check silage of Experiment 1 was of excellent chemical quality, as indicated by low pH, ammoniacal nitrogen and butyric acid values, and high lactic acid values. Slightly lower pH and ammoniacal nitrogen values in the Kylage-treated silage were noted, indicating some possible improvement from the treatment. The check silage of Experiment 2 could be termed *good* but not *excellent* silage, with respect to these criteria. Recirculation of seepage improved the chemical quality only slightly. However, the addition of beet pulp produced an excellent silage and a definite improvement in chemical quality.

The percentages of stored constituents recovered as feedable silage are presented (Table 3). The cornmeal-treated silage as compared to the check silage in Experiment 1 had a greater weight recovery of every constituent

TABLE 3

Per cent of stored constituents preserved as good silage

Treatment	Total fresh weight	Dry matter	Protein	Ether extract	Fiber	N-free extract	Ash	Sugar	Carotene
Experiment 1									
Control	58.8	75.9	73.2	130.4	95.1	68.8	60.6	4.6	88
Kylage	56.6	76.3	73.3	122.6	91.5	72.1	58.6	6.5	87
Cornmeal	65.3	81.5	77.6	120.7	102.5	77.3	65.1	6.0	91
Experiment 2									
Control	58.4	71.2	70.9	135.9	90.5	58.1	74.7	1.6	81
Recirculated	55.4	73.5	69.6	140.7	88.8	64.0	70.7	2.2	80
Beet pulp	78.6	85.0	87.0	149.6	95.8	75.7	95.5	11.2	95

considered, with the exception of ether extract. The addition of beet pulp in Experiment 2 resulted in an even more marked improvement over the check silage, in the recovery rates of all constituents. Relatively small changes in recovery rates resulted from the use of Kylage and the recirculation treatment.

The remaining percentage of stored constituents not accounted for in Table 3 as recovered, represents the total storage loss of each constituent. Total storage losses of dry matter have been subdivided as spoilage, seepage, and fermentation losses (Table 4), fermentation losses being calculated by difference. The extent of dry matter losses through top spoilage was relatively small, being about 0.5% for all silages, since only material stored below the sealing level was considered.

Total storage losses were reduced nearly one-fourth by the addition of cornmeal, as compared to the control silage, reduction of seepage loss being the primary factor involved. However, if all the seepage loss from this silo is

TABLE 4
Distribution of losses in stored dry matter

Treatment	Spoilage	Seepage	Fermentation	Total
		(%)		
Experiment 1				
Control	0.47	13.12	10.52	24.11
Kylage	0.44	11.33[a]	11.92	23.69
Cornmeal	0.37	8.91[a]	9.23	18.51
Experiment 2				
Control	0.40	12.17	16.24	28.81
Recirculated	0.54	11.73	14.26	26.53
Beet pulp	0.27	6.09[a]	8.68	15.04

[a] Calculated on stored forage D.M. only—cornmeal treatment = 13.43%, Kylage, 11.47%, and beet pulp treatment = 9.08% lost in seepage.

attributed to the forage portion of the mixture (see footnote to Table 4), it appears that the percentage loss of forage dry matter through seepage was not reduced. Per cent of stored forage dry matter lost in seepage was lowest for the Kylage treatment in 1954.

Seepage, fermentation, and total losses in the silage mixture were reduced by nearly one-half by the addition of beet pulp in Experiment 2. If all seepage losses from this silo are attributed to the forage stored, seepage losses of forage were reduced by about one-fourth as compared to the control silage. Rather small improvements in loss values resulted from seepage recirculation.

TABLE 5
Results of feeding trial, 1954–1955 and 1955–1956

	1954 Crop			1955 Crop		
	Check	Kylage	Corn-meal	Check	Recircu-lated	Beet pulp
Live weight						
Initial (lb.)	1,074	1,076	1,057	913	902	915
Average (lb.)	1,095	1,094	1,072	932	928	931
Average gain per day (lb.)	1.02	0.98	0.740	1.14	1.29	0.93
Milk production (FCM)[a]						
Initial per cow per day (lb.)	33.67	34.33	34.97	22.85	23.47	23.89
Average per cow per day (lb.)	32.83	33.56	33.36	21.72	21.86	21.74
30-day decline (lb.)	0.88	0.71	2.31	1.65	2.51	2.86
30-day decline (per cent)	2.61	2.07	6.61	7.64	11.76	11.72
Feed dry matter consumed per day per cow						
Silage (lb.)	21.97	21.49	27.66	19.31	19.08	23.93
Concentrates (lb.)	7.41	7.49	0.34	5.09	5.21	0.00
Total (lb.)	29.38	28.98	28.00	24.40	24.29	23.93
Feed dry matter consumed per per day per 100 lb. liveweight						
Silage (lb.)	2.006	1.964	2.580	2.083	2.055	2.584
Concentrates (lb.)	0.677	0.685	0.032	0.549	0.560	0.000
Total (lb.)	2.683	2.649	2.612	2.632	2.615	2.584
Ratio—grain:milk	1:4.4	1:4.5	1:98.1	1:4.3	1:4.2	

[a] Av. days in milk at start of feeding trial 1954 crop, 96 days; 1955 crop, 138 days.

marked in the silages of Experiment 1 and least in the beet pulp treated silage. The silages of both experiments were characterized by typical changes in the percentages of crude constituents in the dry matter. Increases in ether extract and crude fiber, and decreased nitrogen-free extract, were the most marked and consistent. Increases in concentration of carotene and decreases in levels of sugar (which had been largely utilized in the fermentation) were common to all the silages.

The check silage of Experiment 1 was of excellent chemical quality, as indicated by low pH, ammoniacal nitrogen and butyric acid values, and high lactic acid values. Slightly lower pH and ammoniacal nitrogen values in the Kylage-treated silage were noted, indicating some possible improvement from the treatment. The check silage of Experiment 2 could be termed *good* but not *excellent* silage, with respect to these criteria. Recirculation of seepage improved the chemical quality only slightly. However, the addition of beet pulp produced an excellent silage and a definite improvement in chemical quality.

The percentages of stored constituents recovered as feedable silage are presented (Table 3). The cornmeal-treated silage as compared to the check silage in Experiment 1 had a greater weight recovery of every constituent

TABLE 3

Per cent of stored constituents preserved as good silage

Treatment	Total fresh weight	Dry matter	Protein	Ether extract	Fiber	N-free extract	Ash	Sugar	Carotene
Experiment 1									
Control	58.8	75.9	73.2	130.4	95.1	68.8	60.6	4.6	88
Kylage	56.6	76.3	73.3	122.6	91.5	72.1	58.6	6.5	87
Cornmeal	65.3	81.5	77.6	120.7	102.5	77.3	65.1	6.0	91
Experiment 2									
Control	58.4	71.2	70.9	135.9	90.5	58.1	74.7	1.6	81
Recirculated	55.4	73.5	69.6	140.7	88.8	64.0	70.7	2.2	80
Beet pulp	78.6	85.0	87.0	149.6	95.8	75.7	95.5	11.2	95

considered, with the exception of ether extract. The addition of beet pulp in Experiment 2 resulted in an even more marked improvement over the check silage, in the recovery rates of all constituents. Relatively small changes in recovery rates resulted from the use of Kylage and the recirculation treatment.

The remaining percentage of stored constituents not accounted for in Table 3 as recovered, represents the total storage loss of each constituent. Total storage losses of dry matter have been subdivided as spoilage, seepage, and fermentation losses (Table 4), fermentation losses being calculated by difference. The extent of dry matter losses through top spoilage was relatively small, being about 0.5% for all silages, since only material stored below the sealing level was considered.

Total storage losses were reduced nearly one-fourth by the addition of cornmeal, as compared to the control silage, reduction of seepage loss being the primary factor involved. However, if all the seepage loss from this silo is

TABLE 4

Distribution of losses in stored dry matter

Treatment	Spoilage	Seepage	Fermentation	Total
		(%)		
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[a] Av. days in milk at start of feeding trial 1954 crop, 96 days; 1955 crop, 138 days.

Results from the feeding trials are presented (Table 5). Animal response to the rations used in either trial did not differ significantly (analysis of variance) with respect to live weight gains or milk production. The higher silage dry matter consumption per 100 lb. of live weight observed with cornmeal and beet pulp treated silage was highly significant. Little or no additional concentrate was fed with these silages. Total dry matter consumption was not significantly different between groups.

DISCUSSION

Only that material stored below the seal was considered to be experimental, and top spoilage losses for all silos were very low, since the weighted plastic covers formed a good seal. The inclusion of large amounts of top spoilage in total losses of experimental silages is of limited value. Percentage losses in top spoilage are affected markedly by factors such as total amount of material stored, ratio of diameter to height of silo, and type of cover, if any, all of which have no direct bearing on the methods under study. Spoilage was eliminated to the extent possible, for these reasons.

It is apparent that the possibilities of improving the chemical quality of a silage by treatment depend on the quality of the corresponding untreated or control silage. Improvement from treatment can be expected only in cases where no treatment results in a relatively poor quality of silage. Frequent production of untreated silage of excellent quality probably accounts for the reluctance of many farmers to use a preservative of any kind, and for the variable results reported by various investigators. Use of a nonfeedstuff (chemical) preservative presents the distinct possibility of producing no appreciable benefit. However, the residual feeding value of feedstuffs added as preservatives can be expected, even when fermentation characteristics are not improved because of excellent control silage.

Improvement of chemical quality by the Kylage treatment was so small that it should be considered within the range of experimental error. Other data at this station *(6)* and others *(2)* have demonstrated that Kylage will improve the chemical quality when compared to a poor control silage, However, the excellence of the control silage in this experiment made quality improvement by any treatment very unlikely. No explanation for the apparent reduction of seepage losses by Kylage treatment is known. The reduction of total dry matter losses from 24.1 to 23.7% by Kylage treatment, if a real difference, amounts to only 1.5 lb. of dry matter per ton of 18.4% dry matter forage ensiled. Neither did the Kylage treatment produce any significant improvement in feeding value. Therefore, no measurable benefit was obtained from the treatment under these particular conditions.

The value of the cornmeal treatment for improving the chemical quality of fermentation products cannot be assessed from the data of Experiment 1, since the check silage was of excellent quality. It produced no deleterious effects, but was apparently unnecessary. Therefore, if the expense of adding

cornmeal to this particular silage is to be justified it must be on the basis of its contributions to preservation efficiency, palatability, feeding value, and convenience. However, the beet pulp addition in 1955 did improve chemical quality noticeably, as compared to the check silage of that year, which was of lower quality than the control of the previous year.

A comparative evaluation from the standpoint of efficiency of dry matter preservation is presented (Table 6). It will be noted that all seepage losses

TABLE 6
Storage losses of forages and additives

	Material	Stored Dry matter		Storage loss		Recovered Net dry matter
	(lb.)	*(%)*	*(lb.)*	*(%)*	*(lb.)*	*(lb.)*
		1954 Crop				
Forage stored without an additive and cornmeal stored in a bin						
Forage	2,000	17.8	356.0	24.11	85.8	270.2
Cornmeal	200	90.0	180.0	0	0	180.0
Forage stored with added cornmeal						
Forage portion	2,000	17.8	356.0			275.3
Fermentation loss				9.23	32.9	
Seepage loss				13.43	47.8	
Cornmeal portion	200	90.0	180.0			163.4
Fermentation loss				9.23	16.6	
Net gain of treatment						
Forage dry matter + 5.1 lb. Cornmeal dry matter — 16.6 lb.						
		1955 Crop				
Forage stored without an additive and beet pulp stored in a bin						
Forage	2,000	17.4	348.0	28.81	100.3	247.7
Beet pulp	200	87.1	174.2	0	0	174.2
Forage stored with added beet pulp						
Forage portion	2,000	17.4	348.0			286.2
Fermentation loss				8.68	30.2	
Seepage loss				9.08	31.6	
Beet pulp portion	200	87.1	174.2			159.1
Fermentation loss				8.68	15.1	
Net gain of treatment						
Forage dry matter + 38.5 lb.						
Beet pulp dry matter — 15.1 lb.						

in this comparison have been charged to the forage portion of the silage, and fermentation losses charged equally to both parts of the mixed silages. The use of cornmeal in 1954 resulted in a saving of an additional 5.1 lb. of forage dry matter for each ton of green forage stored, but cost 16.6 lb. of cornmeal dry matter. The use of beet pulp in 1955 resulted in a saving of 38.5 lb. of forage dry matter at a cost of 15.1 lb. of beet pulp dry matter, per ton of green forage stored. The cost of these treatments will, of course, depend on price relationships at a particular time and place. It is unlikely that price relationships would be such that the cornmeal treatment could be justified on the basis of forage dry matter preserved; however, such a justification for the beet pulp treatment is conceivable. If this latter treatment is calculated at the fairly reasonable prices of $0.015 per lb. of forage dry matter and $0.034 per lb. of beet pulp dry matter ($60.00 per ton, 87.1% D.M.), the net gain is seven cents per ton of forage ensiled.

The improvement of silage feeding value by these feedstuff additives, measured by improved palatability and reduced requirements for additional supplements, was amply demonstrated in the feeding trial. The economy of this improvement is, however, somewhat questionable. The initial ratio of feedstuff additive to forage was 1:10 on a weight basis, and was used for estimating the cornmeal and beet pulp content of the silages as fed. However, heavy losses of moisture from the forage portion during storage resulted in a much narrower ratio at the time of feeding. The more constant ratio of initial dry matter in the preservative and forage would provide a more accurate estimate of the amount of preservative being fed in such silages.

Calculations of the forage and additive ratios in the silage finally fed (Table 6) indicate that about 37% of the dry matter in the treated silage of Experiment 1 was cornmeal, and that about 36% was beet pulp in the treated silage of Experiment 2.

On the basis of this, the average daily silage dry matter consumption from cornmeal treated silage, 27.66 lb. (Table 5), contained 17.43 lb. of forage dry matter, as compared to 21.97 lb. of forage dry matter consumed from the control silage. Similarly, forage dry matter consumption was 15.32 lb. per day from the beet pulp silage ration as compared to 19.31 lb. from the control silage. The feeding of cornmeal or beet pulp at levels higher than grain feeding for the control groups was, to a large extent, unavoidable, in view of the experimental plan of unrestricted silage consumption. This considerable reduction in forage consumption, as compared to the control silages, would be considered undesirable under most economic conditions, particularly in herds of average production levels. Since these silages contained less than two-thirds of forage dry matter, a ration containing a high proportion of forage could be attained only by feeding another forage simultaneously. The effect on consumption and production of such a feeding system cannot be predicted from these data.

Recirculation of seepage appears impractical from these results. It is possible that improvement of quality from this procedure would be more pronounced if a much lower quality control silage had resulted. However, since the improve-

ment in preservation efficiency is slight and considerable extra equipment is required, use of a preservative would seem more advisable.

CONCLUSIONS

The addition of cornmeal, beet pulp, and Kylage, and seepage recirculation, as treatments for improving rate of nutrient preservation, silage quality, and feeding value were compared with two control silages over a two-year period.

Cornmeal and Kylage treatments produced only slight if any improvement in the excellent quality of the control silage of the first experiment.

Beet pulp treatment produced definite improvement, and seepage recirculation only slight improvement, over the lower-quality control silage of the second experiment.

Neither Kylage treatment nor seepage recirculation produced significant improvements in nutrient preservation or feeding value as compared to their respective controls.

Both cornmeal and beet pulp treatments produced an improved rate of nutrient preservation of the total silage and a marked improvement of feeding value. However, a marked disadvantage with respect to total forage dry matter consumption was also noted.

These experiments indicated that, although certain benefits from the use of these treatments may be expected when compared to poor-quality control silages, the rather limited improvement expected when compared to an excellent or good control silage usually would not be economically justifiable.

The need for an untreated control silage, for each experiment in which evaluation of preservatives is to be made, was demonstrated.

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PRESERVATION OF FORAGE NUTRIENTS AS SILAGE IN GAS-TIGHT ENCLOSURES OF POLYVINYL CHLORIDE PLASTIC [1]

W. L. LARRABEE [2] AND M. A. SPRAGUE [2]

A study was made of nutrient retention of forages, preserved as silage and enclosed in gas-tight wrappers made of polyvinyl chloride plastic film. These wrappers exclude air, limit respiration, encourage desirable fermentation, prevent drying and excessive losses of nutrients, and protect against rain and snow. They provide a method for storing supplemental forage adjacent to silos and in temporary grazing areas. They can be unloaded manually or with power equipment, and can be built in many sizes. Fifteen units have been used on farms, and proved practicable. *Editor.*

This study was undertaken to determine the degree of nutrient retention in forages preserved as silage in gas-tight polyvinyl chloride plastic film.

The upright tower silo has been most extensively used in the United States. Bunkers, stacks, and pits of various sizes, common for many years in Europe, are now frequently found on American livestock farms, also. In many instances, however, savings in construction costs with the more temporary storage units are not fully realized, owing to the high nutrient losses. Extensive respiration, leaching, and aerobic spoilage are difficult to control in most of these structures, consequently, poor-quality silage is a commonplace.

The detailed study of two ten-ton stacks described in this paper followed extensive studies with smaller units, over a period of three years *(9, 10)*.

LITERATURE REVIEW

Nutrient losses have been classified as seepage, surface spoilage, and fermentation. The last-named includes both respiration and fermentation losses collectively. All normally are expressed best as dry matter lost.

Seepage losses reported by investigators vary widely. Allred *et al. (2)* reported from 2.4 to 9.5% total dry matter lost by seepage in 10- × 30-foot silos filled with immature grass forage. Over a period of seven years, Archibald and Gunness *(3)*, using wilted forage, found total dry matter loss due to seepage from 100-ton silos to average only 0.54 per cent. Amount of dry mat-

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[2] Former Research Assistant in Farm Crops, and Professor in Farm Crops, respectively.

ter lost by seepage is directly proportional to the moisture content of the crop being ensiled and internal pressures developed.

Loss due to aerobic microbial activity, commonly referred to as "top spoilage," is of such significant proportion that all investigators in silage preservation take it into consideration. The depth of spoiled layer will vary with porosity of the enclosure and of the silage mass exposed to air. Turk *et al. (12)* report 5.0 and 4.4% dry matter loss in tower silos due to top spoilage alone. Because the two silos used in these studies were in poor repair, there was considerable spoilage around the sides. The combined spoilage on both top and sides amounted to 14.1 and 17.4% dry matter loss, respectively. The only incidence reported heretofore of no surface spoilage in commercial silos is that described by Shepherd *et al. (8)*, in gas-tight silos constructed from steel plates with a glass-like coating. Horizontal silos, with greater proportionate surface area than tower construction, suffer correspondingly higher surface losses. Hendrix *(6)* reports approximately five per cent of the total dry matter losses as surface spoilage in various tower silos and from eight to 16% in various types of trenches and stacks.

Dry matter losses listed as fermentation arise from plant respiration and from enzymatic and microbial activity. In addition to respiration of live plant cells, Watson and Ferguson *(14)* contend that intracellular respiration and plant enzymes further the degradation of plant nutrients into simpler constituents. Allen *et al. (1)* and, more recently, Kroulik *et al. (7)* have identified the classes of organisms common in silage as thermophiles, coliform, obligate anaerobes, and lactobacilli. Agreement is unanimous that proper cultivation of lactobacilli organisms is the key to successful fermentation procedure, with minimum loss of feed nutrients.

Taylor *et al. (11)*, in 1940, considered ten to 15% total loss of dry matter from all sources to be nominal and unavoidable. Shepherd *et al. (8)* in 1953 reported that dry matter losses in tower silos during storage from summer to winter usually ranged between ten and 16%. Allred *et al. (2)*, on the other hand, observed total dry matter losses from 28.1 to 31.9% over a period of two years, in four silos filled each year with immature, unwilted forage. Three silos were treated with sodium metabisulfite, molasses, and brewers grains, respectively, and one had no preservative. Less than one-half the losses were due to top spoilage and seepage.

MATERIALS AND METHODS

During June, 1955, two silage stacks of about ten-ton capacity were constructed of alfalfa (50%), timothy (25%), bluegrass, and weeds, and enclosed within a gas-tight envelope of plastic film. Each stack had a circumference of 32 feet at the base and 24 feet at the top, was tapered slightly to a height of about ten feet. All seepage was collected, measured, and sampled for assays. Thermocouples were placed at the center of each stack one foot from the bottom and one foot from the top, and daily recordings made.

The plastic used in this experiment was a polyvinyl chloride formulation,

KDA-2965 Olive 21, 0.008-inch in thickness and manufactured by the Bakelite Company, Bound Brook, New Jersey. For each stack, a sleeve 26 feet long, with a diameter of 10½ feet, was fabricated. A drain tube was attached six feet from one end and that end was gathered by hand and tied tightly to form a large bag. Starting at the open end, the plastic was rolled down like a stocking and the bag put in place near the ensilage blower.

A section of snow fence, tied to form a ten-foot-diameter ring, was placed around the outside of the rolled plastic and anchored with several metal posts. The plastic was unrolled to the top of the snow-fence form and anchored temporarily.

When the form and plastic was level-full with well-trampled, chopped forage, a second snow-fence ring, eight feet in diameter, was placed immediately above and, as filling and trampling continued, the form was gradually elevated until it reached 18 inches above its original position. The top was rounded off and the upper, smaller form untied and removed from the stack. The plastic was unrolled, pulled over the top, gathered, and tied, the tight closure completing the gas-tight envelope. The lower form was at first left in place as a protective measure, but later was found to be unnecessary.

The forage was chopped in the field when the alfalfa was in full bloom and each load was weighed on a state-certified platform scale. Molasses was applied at the rate of 47.8 pounds per ton to Stack I and 69.8 pounds per ton to Stack II. (Difficulty in regulating the molasses valve resulted in none being applied to the middle layers of Stack I, and accounted for the difference in molasses applied to the two stacks.)

Stack I was opened after 22 weeks of storage and Stack II after 20 weeks. Reliable weight measurements were obtained by forking the silage into wagons as soon as the bag was opened. The entire weighing-out operation for each stack was completed in one day.

Sampling of both molasses and forage was made at the blower at time of filling. Two ½-pint samples of molasses were obtained from each 55-gallon drum used, and later composited for analysis. Small amounts of forage were obtained at about ten-second intervals from the blower hopper. Three- to four-pound samples from each load were placed in a polyethylene bag, frozen, and stored in a freezer to await analysis. Samples of the silage were taken in a similar manner. After each load of silage had been weighed, it was unloaded with conventional equipment; samples were removed from the conveyor hopper at this time. Each stack was unloaded in three nearly equal loads. The six- to eight-pound samples obtained were representative of the top, middle, and bottom third of each stack.

Seepage was measured as it collected in the sunken barrels. Temperature readings were taken once each day for 18 days, after filling with a Rubicon portable potentiometer.

Dry matter content of forage and silage was determined by drying at 70° C. for 36 hours. The pH was determined with a Beckman Model G pH meter equipped with glass and calomel electrodes. Total titratable acidity and volatile

bases and acids were determined by the method of Foreman *(5)*, as modified by Woodman *(16)*. Methods of the A.O.A.C. *(4)* were used for the proximate analyses. Dry matter in seepage was determined initially by drying a 20-ml. aliquot on a steam table and finally drying in a vacuum oven at 28 inches of mercury and 70° C.

RESULTS

Within 15 minutes after sealing, inflation of the plastic was observed. Internal gas pressures appeared to reach a maximum about 24 hours after filling (Figure 1), after which pressures within the bag diminished gradually. Near-atmospheric levels were reached two weeks from the time of filling.

The gas produced was presumably carbon dioxide, formed as a by-product of respiration. During inflation, the plastic stretched about 25 per cent without showing signs of weakening or undue stress. Manufacturers' specifications characterize the vinyl film as having an ultimate elongation of 380 per cent, with a tensile strength of 2,300 lb. per square inch. Permeability of the film to carbon dioxide is relatively high (348 gm. per 100 square inches per day, at atmospheric pressure and 100% relative humidity), which accounts for the observed loss of the internal pressure. Permeability of the film to other gases and moisture vapor is relatively low, and little if any oxygen is believed to enter the bag during the period of fermentation and storage.

Temperature relations. The immediate rise in temperature at the top and bottom levels of Stack I indicates rapid initial respiration of the green forage (Figure 2). However, respiration ceased abruptly after the second day. Maximum temperatures were reached on the sixth day after filling, and are considered favorable for desirable fermentation.

FIG 1. Respiratory gases collect within the plastic within 17 hours of closure. Vinyl film, permeable to carbon dioxide, permits collapse within two weeks.

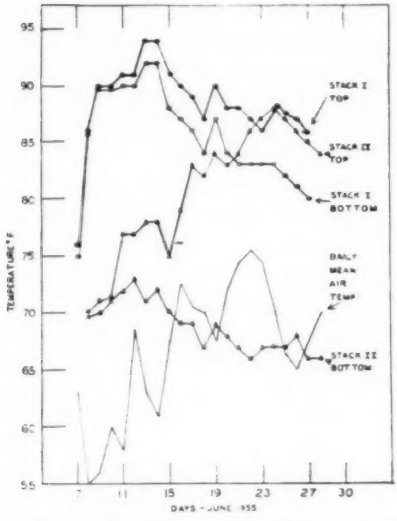

FIG. 2. Daily mean temperatures at the top and bottom of ten-ton stacks of silage, in plastic and mean air temperature during 20 days after filling.

Temperatures recorded in Stack II do not show as great an increase during the first week as those in Stack I. Temperatures of the top of Stack II conform closely to the daily mean air temperature. Possibly, the thermocouple placed at a depth of 18 inches in the forage became dislodged to a more shallow depth, where it reflected outside temperature variations. The temperature of the bottom of both stacks was consistent.

Respiration in Stack II was apparently more effectively controlled by more thorough packing at the time of filling, perhaps aided by the greater quantity of molasses added.

The favorable temperatures observed in both stacks were a direct result of the effective exclusion of air by the plastic film.

Acceptability as feed. When the stacks were opened, there was no evidence of any surface spoilage. The silage was a uniform dark green–brown color throughout both stacks. When fed to yearling beef steers, its acceptance was excellent, with a consumption rate in excess of 55 pounds per animal per day.

Seepage losses. Moisture content of the forage ensiled in Stacks I and II was 77.4 and 75.8%, respectively, and only 180 and 88 gallons of seepage were collected, respectively.

Stack I lost more than twice as much protein and nearly twice as much dry matter in the seepage as did Stack II (Table 1). These total losses are lower than generally reported in the literature, perhaps because the total fluid loss was lessened by the limited confinement and resulting low pressures in the stacks.

TABLE 1

Weight of forage and molasses ensiled, and losses of dry matter and protein in the seepage from field-size stacks

Stack	Forage and molasses			Seepage losses			
	Ensiled	Dry matter	Protein	Dry matter		Protein	
	(lb.)	*(lb.)*	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*	*(%)*
I	22,260	5,306	738	129	2.6	31	4.2
II	20,905	5,421	727	74	1.5	14	1.9

Total dry matter losses. Total losses of dry matter during storage were extremely low (Table 2).

Stack I, which had reflected higher temperatures and greater seepage than Stack II, similarly suffered greater total dry matter losses.

The total loss of protein from Stack II was less than one-third that from Stack I. In these determinations no correction was made for volatile bases. Because protein analyses were made on oven-dried materials, they do not reflect the volatile nitrogen in the silage.

Thirty-one of the 53 pounds of protein (60%) lost from Stack I were recovered in the seepage. Under the more efficient fermentation in Stack II, 14 of the 17 pounds of protein (83%) lost were recovered in the seepage (Table 1). The use of a greater quantity of molasses in Stack II no doubt influenced these losses.

The loss of dry matter attributed to fermentation in Stack II was only one-half as much as that in Stack I (Table 3). The greater flow of seepage from Stack I resulted in a greater percentage of dry matter lost in this way. Absence of any surface spoilage within the container may be attributed to exclusion of oxygen by the plastic.

Chemical composition. The high pH observed half-way up in Stack I is reflected in higher volatile base and volatile acid determinations at that level (Table 4). The smaller applications of molasses applied to the forage in the middle of the stack may account for these differences. An odor of butyric acid was detected in these middle layers of silage, which may account for the greater quantity of volatile base. Putrefying bacteria may be expected to increase the amount of ammonia in silage *(13)*.

The analyses for pH, volatile base, and volatile acid clearly indicate that

TABLE 2

Total losses of dry matter and protein from field-size stacks covered with vinyl plastic

Stack	Forage and molasses ensiled		Silage losses			
	Dry matter	Protein	Dry matter[a]		Protein	
	(lb.)	*(lb.)*	*(lb.)*	*(%)*	*(lb.)*	*(%)*
I	5,306	738	435	8.2	53	7.2
II	5,421	727	235	4.3	17	2.3

[a] Corrected for volatile acids.

TABLE 3

Origin of dry matter loss from forage stored in gas-tight, plastic-covered, field-size stacks

Stack	Lost in seepage	Lost as surface spoilage	Fermentation loss	Total loss
	(%)			
I	2.6	0.0	5.6	8.2
II	1.5	0.0	2.8	4.3

the silage underwent a more desirable fermentation in the stack in which more molasses was used (Stack II). The pH of the silage decreased steadily from top to bottom, as did volatile bases and volatile acids. The amounts of volatile bases in composite samples from Stacks I and II were similar, indicating nearly similar ammonia formation in the two stacks.

DISCUSSION

Respiration with resultant temperature rise is dependent upon available oxygen. Compaction of the forage at the time of filling is a means of initial control. In this experiment, the additional molasses applied to Stack II is believed to have bound the forage better than Stack I, for more effective packing. Sealing of the silage mass in an air-tight envelope served to maintain the established anaerobic atmosphere.

The moisture content of the ensiled crop and the magnitude of the pressures developed determine the extent of seepage flow. It would seem reasonable to expect, therefore, that silage masses of equal moisture content but of less height would lose less through seepage than those under the greater pressures of tall silos. Losses from seepage may be reduced by lowering the moisture content of the crop by wilting, although the wilting process is likely to introduce poorer exclusion of air and additional respiration losses.

The absence of surface spoilage on silage stored in gas-tight containers materially reduces total losses. The use of plastics described here provides a

TABLE 4

Acidity, volatile bases, and acids at top, middle, and bottom, and in composite samples of silage in field-size stacks covered with vinyl plastic

	Stack	pH	Titratable acidity[a] ($\frac{N}{10}$ NaOH)	Volatile bases[a] ($\frac{N}{10}$ H_2SO_4)	Volatile acids[a] ($\frac{N}{10}$ NaOH)
			(ml.)		
I	Top	3.80	438.8	28.0	101.7
	Middle	4.10	418.6	45.7	167.4
	Bottom	4.00	418.5	36.9	143.5
	Composite	3.90	423.5	37.8	123.8
II	Top	3.90	474.8	43.3	117.1
	Middle	3.80	486.9	32.5	112.3
	Bottom	3.65	512.7	27.8	82.9
	Composite	3.80	503.0	34.4	105.8

[a] For 100 gm. silage.

means of storing supplemental forage without experiencing the usually large spoilage losses found in pits, bunkers, or paper-lined snow-fence silos.

The fact that some butyric acid was found in the middle of Stack I, which received less molasses, indicates the value of using adequate amounts of preservatives.

The levels of total dry matter losses (8.2 and 4.3%) in the two ten-ton stacks described here are sufficiently low to suggest that large field-sized stacks may be efficiently preserved under a gas-tight plastic cover. Watson and Ferguson *(15)* state that eight to ten tons of silage, where nutritive losses are to be measured, approximate field practice.

A second phase of this study was to determine the effectiveness of stacks enclosed in plastic as used in actual farm practice. During 1955 and 1956 a total of 15 stacks were put up, each containing from 30 to 75 tons of forage, six at the Experiment Station and nine on private farms throughout New Jersey. Two bags have been used a second season and are in good repair for a third use. Forages ensiled have included alfalfa, corn, soybeans, sorghum, oats, peas, and several grass-legume mixtures.

The sleeves of polyvinyl chloride plastic film were fabricated and supplied by the Bakelite Company and all were 20 feet in diameter. The 15 stacks of settled silage ranged in height from eight to 15 feet, depending on the amount of forage available (Figure 3).

In all cases where air-tight enclosures were maintained, aerobic spoilage was completely lacking or of a negligible amount. When the stacks were opened, the silage surface was of the same good color and odor as the entire mass. The excellent silages, as judged on opening and feeding, indicated that favorable fermentations had taken place. Farmer-reaction, therefore, has been consistently favorable.

FIG. 3. Two stacks (of 53 and 67 tons' capacity) of grass silage in plastic at the Dairy Research Station, Beemerville, N. J.

On a few occasions, bags have been damaged by vandals. Although the plastic is strong and can be handled easily with ordinary care, when punctures are caused by sharp tools subsequent spoilage occurs in proportion to the size of the opening and time before reclosure. Small tears and punctures may be repaired easily with pressure tape or solvent-applied patches.

In addition to providing a good means of storing supplemental forage with low nutrient losses, applying plastic film to silage stacks can serve to make feeding of livestock more flexible. Because the only requirement for the placement of these structures is that the ground be reasonably level, they can be built in pastures of excess spring growth for use in summer or post-grazing periods; in areas commonly distant to the barn for feeding young stock, or adjacent to existing silos to increase silage storage space as needed. Unloading may be accomplished by hand or with bulk-handling power equipment. Different-sized units may be chosen to suit both the forage supply and livestock needs.

The waterproof plastic cover not only prevents drying out of the silage but also sheds rain and snow which may contribute to excessive leaching of valuable nutrients.

SUMMARY AND CONCLUSION

A new method of ensiling forages on the farm is proposed, and two field-size units were used in detailed studies to test their efficiency in preservation of feed nutrients. A gas-tight cover of polyvinyl chloride plastic film was used to enclose stacked forage, exclude air, limit respiration, and encourage desirable fermentation. Fifteen farm units substantiate its practicability for use on farms.

A high-quality silage was produced, with consistently low seepage and fermentation losses, and no surface spoilage. The method has been tested with all species commonly used for silage in the Northeast. All the basic principles of silage-making are effectively employed, to hold nutrient losses to a minimum and produce a high-quality product.

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UTILIZATION OF BLACKSTRAP MOLASSES, UREA IN MOLASSES, AND AMMONIATED MOLASSES BY DAIRY HEIFERS [1]

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Dairy heifers were fed molasses, urea-molasses, and ammoniated molasses. The molasses were fed with corn silage and minerals, and cottonseed meal supplied protein in all rations except those containing high levels of nitrogenous additives. Crude protein was more digestible in rations containing five per cent urea and ten per cent urea-molasses, with nitrogen balances of +15.26 gm. and −0.73-gm. per day, respectively. Crude protein in the 15-% ammoniated ration was less digestible than that in the other rations, the nitrogen balance being −5.87 gm. per day. Blackstrap molasses depressed the digestibility of crude protein. Calcium and phosphorus metabolism was satisfactory. *Editor.*

Experimental results have shown that blackstrap molasses in the ration of cattle is worth 67% *(13)* to 100% *(4)* as much as corn. Davis *et al. (5)* have found that when molasses was fed to dairy heifers in amounts up to six pounds per day, the total digestible nutrients appeared to be as available as those furnished by corn. Bray *et al. (3)* showed that the most efficient amount of molasses to feed was three to four pounds per head daily.

Reid *(15)* has recently reviewed the use of urea as a protein replacement. Urea mixed in molasses and fed with low-protein basal rations was utilized well by steers *(7, 20)*. Soybean meal *(7)* and cottonseed meal *(20)*, however, were found to be somewhat superior to urea. Other research *(2, 12)* has shown that a better utilization of urea was obtained from rations containing starch, than from those containing sugars.

Knodt *et al. (11)* have obtained gains of approximately two pounds per day with calves 12 to 16 weeks of age fed ammoniated molasses. Parham *et al. (14)* and Richardson *et al. (16)* obtained gains of 1.04 lb. and 1.18 lb. per day, respectively, when ammoniated molasses was fed to supply part of the required protein. Tillman and Swift *(21)* compared ammoniated molasses and urea to soybean meal as sources of nitrogen for sheep. A highly significant decrease in coefficient of digestibility of the crude protein fraction was found when ammoniated molasses was fed. Working with steers, Tillman *et al. (19)* found only a small difference in digestibility of the crude protein, when a ration con-

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taining cottonseed meal was compared with one in which ten per cent of the nitrogen of the concentrate was supplied by ammoniated molasses.

In vitro studies *(6, 8, 18)* have shown rather poor utilization of the nitrogen in ammoniated molasses. Hershberger *et al. (8)* found that less than one-third of the nitrogen in ammoniated inverted molasses was available to sheep. Davis *et al. (6)* compared ammoniated molasses with soybean meal and found that the nitrogen of the molasses was significantly less digestible (five-per cent level) than that of soybean meal. In Davis' work, dairy steers fed ammoniated molasses retained an average of 16 gm. of nitrogen daily.

King *et al. (9, 10)* found that yearling dairy heifers fed corn silage, 3.0 lb. of molasses containing five-per cent urea, and 1.0 lb. of cottonseed meal gained 1.51 lb. daily, as compared to 1.71 lb. daily for control heifers fed corn silage, 3.0 lb. molasses, and 2.0 lb. cottonseed meal. Only 0.92-lb. gain per day was obtained when heifers were fed corn silage, 3.0 lb. of 15-% ammoniated molasses, and 1.0 lb. of cottonseed meal. When cottonseed meal fed to heifers receiving ammoniated molasses was increased from one to two pounds daily, the average daily gain was 1.90 lb.

Results *(9)* obtained from these feeding trials made it apparent that more exacting studies should be conducted to determine the utilization of molasses, urea-molasses, and ammoniated molasses. This report presents results of digestion trials and balance studies with dairy heifers fed molasses, urea in molasses, and ammoniated molasses. Seven trials were conducted over a two-year period, 1954 through 1955.

EXPERIMENTAL PROCEDURE

Dairy heifers approximately two years old and ranging in weight from 800 to 1,000 lb. were fed corn silage as the basic roughage. The heifers had been bred and were in the third to fourth month of gestation. In all trials except the control without molasses, 3.0 lb. of molasses was fed by mixing with the silage. Cottonseed meal (hydraulic) was fed in varying amounts, according to the type of molasses included in the ration. The heifers were fed sufficient corn silage, molasses, minerals, and protein supplement to meet the requirements as recommended by Morrison *(13)*. The average daily feed consumption and average live weights of the dairy heifers by periods and rations are presented (Table 1).

Twenty gm. of dicalcium phosphate (22.7% calcium and 18.3% phosphorus) and 20 gm. of trace-mineralized salt were fed daily to each heifer in the two control groups (1955). Heifers in the other groups received 50 gm. of dicalcium phosphate and 20 gm. of trace-mineralized salt per day.

The silage was produced from well-eared corn. The ammoniated molasses containing 15% and 32% crude protein equivalent were obtained from a commercial source. The 32% molasses was inverted ammoniated blackstrap. The two kinds of molasses containing urea were prepared experimentally by stirring the urea in the molasses until it was dissolved and uniformly mixed. The chemical composition of the feeds used in the digestion and balance studies is presented (Table 2). Analyses were made according to the A.O.A.C. methods *(1)*.

TABLE 1

Average daily feed consumption and live weights of dairy heifers during the digestion trials and belance studies

Trial	Type of ration	Av. feed consumed daily					
		Corn silage	Cottonseed meal	Molasses	Urea-molasses	Ammon. molasses	Av. live weight
		(*lb.*)					
	Trials conducted in 1954 (3 heifers each)						
1	Control with molasses	53.07	2.0	3.0	0	0	970
2	5-% urea-molasses	50.77	1.0	0	3.0	0	964
	Trials conducted in 1955 (3 heifers each)						
3	Control without molasses	46.60	2.0	0	0	0	860
4	Control with molasses	43.66	2.0	3.0	0	0	922
5	10% urea-molasses	46.42	0	0	3.0	0	914
6	Ammoniated molasses (15% crude protein)	46.37	1.0	0	0	3.0	854
7	Ammoniated molasses (32% crude protein)	45.86	0	0	0	3.0	851

The trials were standard total collection periods of ten days each. Preliminary periods were ten days or longer. Three heifers were used in each trial.

The urea in the five-per cent urea-molasses supplied 20.3% of the total nitrogen of the ration; with the ten-per cent urea-molasses ration, the nitrogen supplied by urea averaged 43.0%. Nitrogen in the ration supplied by the 15-% ammoniated molasses averaged 23.8%; whereas, it was 47.0% for the 32-% inverted ammoniated molasses ration. The urea content of the five-per cent urea-molasses ration averaged 0.93-% of the dry matter: whereas, the ten-per cent urea-molasses ration averaged 2.09%. The sulfur-to-nitrogen ratio in the urea-molasses and the ammoniated molasses rations ranged from 1:8 to 1:12.

Data on digestibility of the components of the rations were analyzed by the method of analysis of variance described by Snedecor *(17)*.

RESULTS AND DISCUSSION

Results of the digestion trials are presented (Table 3). Since the data are related, results of both years' work are presented in the same table. No direct comparisons of coefficients of digestibility should be made between trials of the two years, when the differences are small. This is owing to the fact that two different years' production of corn was used for making the silage.

Coefficients of digestibility of dry matter were similar in most of the rations. The digestibility of the dry matter of both ammoniated molasses rations was significantly lower (five-per cent level) than that of the ten-per cent urea-molasses ration. In the rations which contained urea-molases, the crude protein fraction was digested more efficiently than in the "controls with molasses." In the 1955 trials, the greater digestibility of protein in the urea-molasses ration was highly significant (one-per cent level) when compared to the control ration with mo-

TABLE 2

Average chemical composition of composite feed samples used in the digestion trials and balance studies

Trial	Feeds	Dry matter	Crude protein	Ether extract	Crude fiber	NFE	Ash	Ca	P
		(%)							
1	Corn silage (1954)	24.99	1.91	0.89	6.65	14.34	1.20	0.095	0.040
2	Corn silage (1954)	25.93	2.00	0.79	6.50	15.36	1.28	0.090	0.036
3	Corn silage (1955)	25.79	2.30	0.55	6.12	15.58	1.24	0.109	0.043
4	Corn silage (1955)	27.06	2.41	0.80	6.22	16.31	1.32	0.112	0.039
5	Corn silage (1955)	27.03	2.17	0.43	6.88	16.41	1.14	0.097	0.039
6	Corn silage (1955)	26.66	2.19	0.68	6.96	15.61	1.22	0.119	0.055
7	Corn silage (1955)	27.32	2.33	0.83	6.76	16.17	1.23	0.117	0.036
1	Cottonseed meal (1954)	91.72	41.19	4.48	12.49	26.93	6.63	0.316	0.987
2	Cottonseed meal (1954)	86.93	41.69	4.20	11.76	23.52	5.76	0.333	0.969
3	Cottonseed meal (1955)	91.18	40.88	4.86	10.40	29.92	5.12	0.429	0.895
4	Cottonseed meal (1955)	91.90	41.38	5.55	12.55	27.45	4.97	0.557	0.923
6	Cottonseed meal (1955)	91.26	41.13	5.39	10.84	29.10	4.80	0.429	0.895
1	Molasses (1954)	74.40	4.31	0	0	62.30	7.79	0.904	0.050
4	Molasses (1955)	78.70	4.88	0	0	65.87	7.95	1.023	0.059
2	5% urea-molasses (1954)	74.14	17.00[a]	0	0	50.07	7.07	0.809	0.053
5	10% urea-molasses (1955)	76.31	33.38[a]	0	0	35.82	7.11	0.997	0.045
6	Ammoniated molasses (15%) (1955)	72.06	14.88[a]	0	0	49.51	7.67	1.135	0.115
7	Ammoniated molasses (32%) (1955)	71.19	32.19[a]	0	0	32.10	6.90	1.109	0.187

[a] Crude protein equivalent (N×6.25).

lasses. A comparison of the other fractions showed no consistent differences between the urea-molasses and blackstrap molasses rations.

The greatest significant difference is found in a comparison of the coefficients of digestibility of crude protein of the two ammoniated molasses rations with those of the other rations. Coefficients of digestibility of the crude protein averaged 37.97% for the 15% ammoniated molasses ration and 42.08% for the 32% ammoniated molasses ration. Further calculations using the difference technique indicate that the nitrogen in the ammoniated molasses was completely indigestible in these trials.

Apparently, blackstrap molasses depressed digestibility of the crude protein of the rations. Coefficients of digestibility of the crude protein of the control ration without molasses were significantly higher (one-per cent level) than those of the control ration with molasses.

Digestibility of molasses was calculated by difference, by applying the data obtained in 1955 from the two control rations. The coefficients of digestibility averaged 75.1% for the dry matter and 90.3% for the nitrogen-free extract. The total digestible nutrients on dry-matter basis averaged 75.6% for the molasses.

A more critical examination of the protein metabolism of heifers fed the various molasses rations was made by nitrogen-balance studies. Data are pre-

TABLE 3

Average coefficients of apparent digestibility of experimental rations

Trial	Type of ration[a]	Dry matter	Crude protein	Ether extract	Crude fiber	NFE
		(%)				
		Trials conducted in 1954				
1	Control with molasses	62.10	50.99	74.20	53.10	68.98
2	5% urea-molasses	61.60	57.10	74.68	49.85	68.55
	L.S.D., 5% level	3.83	7.17	12.14	1.93	7.41
	L.S.D., 1% level	8.84	16.55	28.00	4.45	17.08
		Trials conducted in 1955				
3	Control without molasses	66.40	59.73	77.55	60.96	72.43
4	Control with molasses	67.80	50.97	79.12	58.27	75.92
5	10% urea-molasses	68.75	60.44	60.70	64.68	75.31
6	Ammoniated molasses (15%)	65.32	37.97	72.66	62.44	74.08
7	Ammoniated molasses (32%)	64.91	42.08	76.52	65.50	71.73
	L.S.D., 5% level	3.01	4.73	5.04	5.07	3.30
	L.S.D., 1% level	4.38	6.88	7.33	7.37	4.81

[a] See Table 1.

sented (Table 4). Heifers fed the control rations containing 2.0 lb. of cottonseed meal per day, both with and without molasses, showed a positive balance of nitrogen, with a range averaging from 3.65 gm. to 10.69 gm. per day. Excellent retention of nitrogen was found with the heifers fed the five-per cent urea-molasses ration containing one pound of cottonseed meal per day.

With the ration of ten-per cent urea-molasses, a loss of 0.73-gm. of nitrogen per day was found. Two of the three heifers showed a small loss. This less efficient use of urea when fed at high levels (2.09% of the dry matter of the ration) checks with the lower live weight gains obtained in the feeding trials reported earlier *(9)*. In each case where urea-molasses rations were fed, a higher

TABLE 4

Average daily nitrogen balances of heifers in molasses experiments

Trial	Type of ration[a]	Nitrogen intake	Nitrogen output		Nitrogen digested	Nitrogen balance
			Urine	Feces		
		(gm.)	(gm.)	(gm.)	(%)	(gm.)
		Trials conducted in 1954				
1	Control with molasses	142.37	61.86	69.82	50.99	+10.69
2	5-% urea-molasses	140.66	65.08	60.32	57.10	+15.26
		Trials conducted in 1955				
3	Control without molasses	137.07	78.08	55.34	59.73	+ 3.65
4	Control with molasses	146.87	69.55	71.95	50.97	+ 5.37
5	10% urea-molasses	145.63	88.77	57.59	60.44	− 0.73
6	Ammoniated molasses (15%)	135.81	57.56	84.12	37.97	− 5.87
7	Ammoniated molasses (32%)	143.61	55.20	83.23	42.08	+ 5.18

[a] See Table 1.

coefficient of digestibility of the crude protein fraction was found, but there was also a greater loss of nitrogen in the urine.

Heifers fed the ammoniated molasses rations showed low coefficients of digestibility of the crude protein, with high excretion of nitrogen in the feces and low excretion in the urine. This observation confirms the apparent lack of utilization of the nitrogen in the ammoniated molasses by the rumen microorganisms. With the 15-% ammoniated molasses ration, the heifers showed a loss of nitrogen averaging 5.87 gm. per day. In spite of low utilization of the nitrogen of the 32-% ammoniated molasses ration, however, the heifers showed a retention of 5.18 gm. per day. This may have been due to the slightly higher digestibility of the nitrogen of the ammoniated inverted molasses, or to the fact that the heifers had become more adjusted to lower nitrogen absorption. The same heifers were fed the two ammoniated molasses. At the beginning of the trial with the 32-% ammoniated molasses, the heifers had been fed an ammoniated molasses for 33 days. Other evidence, that the problem is not simply one of the rumen microorganism requiring time to become adjusted to the ammoniated molasses, is shown by the failure of yearling heifers *(9)* to utilize the nitrogen in an 84-day feeding period. This utilization of the ammoniated molasses is lower than that found by Davis *et al. (6)*. It is quite clear that at least some batches of ammoniated molasses contain nitrogen in a form unavailable to the ruminant.

A summary of the daily calcium and phosphorus balances of the heifers fed the various rations is presented (Table 5). The rations used in each trial did not seem to influence the retention of these mineral elements, except with the five-per cent urea-molasses ration. The heifers fed this ration lost an average of 3.51 gm. of calcium per day. In this case, a larger amount of calcium was found

TABLE 5

Average daily calcium and phosphorus balances of heifers in molasses experiments

Trial	Type of ration[a]	Calcium intake	Calcium output		Calcium balance	Phosphorus intake	Phosphorus output		Phosphorus balance
			Urine	Feces			Urine	Feces	
		(gm.)							
		Trials conducted in 1954							
1	Control with molasses	51.05	1.30	45.13	+4.62	25.78	0.25	22.24	+3.29
2	5% urea-molasses	48.66	0.77	51.40	−3.51	20.14	0.31	18.65	+1.18
		Trials conducted in 1955							
3	Control without molasses	31.43	2.12	24.55	+4.76	20.83	0.44	14.72	+5.67
4	Control with molasses	45.75	1.45	36.95	+7.35	20.44	0.54	15.78	+4.12
5	10% urea-molasses	45.29	1.27	40.74	+3.28	17.91	0.55	15.00	+2.36
6	Ammoniated molasses (15% crude protein)	53.68	1.28	41.53	+10.87	26.45	0.49	17.94	+8.02
7	Ammoniated molasses (32% crude protein)	49.69	1.27	40.34	+8.08	19.05	0.35	15.72	+2.98

[a] See Table 1.

in the feces than would be expected. The ten-per cent urea-molasses ration did not show this same relationship.

SUMMARY

A series of seven digestion trials and balance studies was conducted with dairy heifers, to study the utilization of molasses, urea-molasses, and ammoniated molasses. The molasses were fed with corn silage and minerals. Cottonseed meal was fed to supply additional protein, in all rations except those containing the higher levels of nitrogenous additives. With the exception of the control without molasses ration, 3.0 lb. of molasses was fed daily to each heifer. A higher coefficient of digestibility of the crude protein fraction was found with the rations that included five-per cent urea and 10-per cent urea-molasses. The nitrogen balance studies of the two rations showed +15.26 gm. and −0.73-gm. per day, respectively.

The crude protein fraction of the rations containing the ammoniated molasses was significantly (one-per cent level) less digestible than that of the other rations. A nitrogen balance of −5.87 gm. per day was found with the 15-% ammoniated molasses rations.

A comparison of the control rations showed a significant depression of digestibility of the crude protein of the ration containing blackstrap molasses. The molasses was found to contain 75.6% total digestible nutrients on the dry-matter basis.

In general, calcium and phosphorus metabolism of the heifers fed the various rations was satisfactory.

ACKNOWLEDGMENTS

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A NUTRITIONAL CONCEPT OF THE GERMICIDAL PROPERTY OF RAW MILK

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Streptococcus pyogenes **was used to study the germicidal property of milk and it was classified as sterilized, inhibited, and uninhibited in different media. Glutathione, mercaptoacetic acid, stannous chloride, sulfanilamide, sodium sulfite, thiamine, ascorbic acid, and hydroxylamine eliminated the lethal effect of unheated whey, but did not permit growth of** *S. pyogenes*. **This organism grew in unheated whey containing meat infusion and yeast extract broths and in that containing albumin heated to liberate sulfhydryls. The lethal effect of raw milk and whey for** *S. pyogenes* **is probably due to a deficiency of free sulfhydryl groups and denatured albumin.** *Editor.*

Usually the streptococci and lactobacilli show, in raw milk, an extended period of bacteriostasis, sometimes even decreasing in numbers before beginning to multiply. Eventually these organisms achieve the same population in raw milk as in autoclaved milk. Still other microorganisms, which exhibit rapid growth in autoclaved milk, decrease steadily and eventually die out in raw milk. This property of raw milk is now generally attributed to a "substance," lactenin, named by Jones and Simms (*16*) and defined in terms of its effect on Group A streptococci.

Jones and Little (*14*) reviewed the literature to 1927. More recent investigations (*3*, *23*) have shown that several conditions, other than heating, may eliminate the germicidal property of milk. Efforts toward identifying a specific compound as the cause of the germicidal property have made slow progress. It has been considered many times, but only minor consideration has been given to a nutritional deficiency aspect of raw milk.

The germicidal property of milk has been detected with many test microorganisms, but *Streptococcus pyogenes* is most favored for this study, because it is the only microorganism known to be sterilized when subcultured in raw milk or whey (*14*). This microorganism has a requirement for heated ovalbumin and free sulfhydryl groups (*21*). Several investigators (*5*, *9*, *10*, *17*, *19*) have reported the appearance of free sulfhydryl groups in milk and whey heated at 75° C. or above, for various periods of time, but prolonged heating may result in decreased concentrations of free sulfhydryl groups *(11)*. Others *(1, 12, 14)* have reported that the germicidal property of raw milk or whey is eliminated

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for *S. pyogenes* when the medium is heated at 80° C. for 20 minutes, 74° C. for 20 minutes, or 100° C. momentarily. These data from unrelated investigations indicate a relationship between free sulfhydryl groups and the germicidal property of milk for *S. pyogenes*. This study [a preliminary report was presented earlier, (*8*)] is concerned with exploring more fully the significance of a possible nutritional deficiency of raw milk as a cause of the germicidal property of milk.

MATERIALS AND METHODS

(1.) *Sterile raw whey*: Fresh, two-hour-old, raw milk from three or four cows of the Cornell University herd was tempered to 40° C. and clotted with commercial rennet extract. The resulting curd was cut and the whey was drained through cheese cloth, after which it was centrifuged in an International, size 1, type C centrifuge for 30 minutes. Residual fat was removed with an aspirator and the whey was decanted from the remaining sediment. The whey was twice filtered through Whatman No. 42 paper and passed through a sterile Selas 0.02 bacteriological filter candle (autoclaved after charging with *N*/10 hydrochloric acid). Sterile whey was aseptically removed to sterile containers in the desired amounts. Heated whey was prepared by placing tubes of sterile raw whey in a water bath maintained at the desired temperature.

(2.) *Whey fractionation*: Raw whey was obtained as in (1.) and half-saturated with ammonium sulfate. The precipitate was centrifuged out and continuously dialyzed, with agitation, against distilled water until salt-free, as shown by addition of barium chloride to the dialysate. Dissolved in a 2-per cent sodium chloride solution, this first precipitate constituted a concentrated lactoglobulin fraction. The remaining supernatant was then fully saturated with ammonium sulfate and centrifuged, after which the wet precipitate of lactalbumin was dialyzed in a manner similar to the first fraction and diluted with distilled water to yield a lactalbumin concentrate of the desired strength. Both fractions were sterilized as in (1.).

(3.) Ovalbumin was prepared from fresh egg white by repeated precipitation with ammonium sulfate. Solutions containing 20 mg. per milliliter were sterilized as in (1.).

(4.) Bacterial cultures [2] were grown in meat infusion broth for approximately 18 hours at 37° C.

(5.) Meat infusion broth consisted of 50% by volume of meat infusion, made to volume with distilled water, after addition of 1-% tryptone, 0.2-% dipotassium phosphate, and 0.1-% glucose.

(6.) Concentrated raw whey was obtained by pervaporation and sterilized as in (1.).

(7.) The nitroprusside test of Josephson and Doan (*17*) was used for the estimation of free sulfhydryl groups.

[2] From the Laboratory of Bacteriology, Stocking Hall, Cornell Univ., Ithaca, N. Y., and designated as follows: *Streptococcus pyogenes*—A, A1, A3, A4, A8, A9, A11, and C203S. *Streptococcus agalactiae*—67A. From the American Type Culture Collection, 2029 M. St., N. W., Washington 6, D. C., and designated as follows: *Micrococcus pyogenes* var. *aureus*–6538P and *Escherichia coli*–8739.

TABLE 1

Components of an essentially chemically defined medium and optimum concentrations for growth of Group A Streptococcus

Component	Amount necessary for optimal growth in 10 ml. of medium (*18*)
	(γ)
Glucose	25
Glutamine	500
Tyrosine	1,000
Tryptophane	500
Uracil	100
Adenylic Acid	100
Thioglycollic Acid	1
Glutathione	4
Thiamine	0.01
Nicotinic Acid	1
Pantothenic Acid	10
Riboflavin	0.04
Vitamin B_6	20
Biotin	1

(8.) Cysteine and *p*-chloro-mercuribenzoate solutions of 10^{-4} molar concentrations were sterilized by filtration.

(9.) Components of the semi-synthetic defined medium (Table 1) of Pappenheimer and Hottle (*18*) were prepared and sterilized as recommended. Solutions were of such strength that when they were incorporated into test media, optimum concentrations were secured. Reducing agents (Table 2) were sterilized by filtration.

(10.) Slides were prepared from test cultures according to common procedures and stained with methylene blue. Plate counts were made on meat infusion agar to which 10% horse blood was added to facilitate counting, and incubated at 37° C. for 36 hours.

EXPERIMENTAL PROCEDURES

Procedures for evaluating the thermogenic stimulatory property of milk. *E. coli*, a microorganism which grows well in simple media; *S. pyogenes*, which grows well only in complex media, and *M. pyogenes* var. *aureus* and *S. agalactiae*, microorganisms which grow well in media between the two extremes, were culti-

TABLE 2

Concentration of reducing agents added to raw whey

Reducing agent added	Concentration
	(%)
Glutathione	0.01
Mercaptoacetic Acid	0.025
Stannous chloride	0.005
Sulfanilamide	0.005
Sodium sulfite	0.004
Thiamine	0.0005
Ascorbic Acid	0.01
Hydroxylamine	0.0005

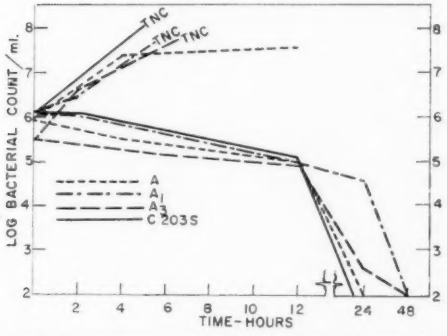

FIG. 1. The growth of *Streptococcus pyogenes*, A, A1, A3, and C203S in whey heated at 100° C. for five minutes (four ascending lines), and in raw whey (four descending lines). TNC = too numerous to count.

vated in raw and heated whey. Growth curves were constructed from plate counts, and stained slides were prepared for each culture.

Growth of *S. pyogenes* in dialyzed meat infusion broth—a nutritionally deficient medium—was compared with growth in raw, sterile whey and with growth in raw, sterile whey to which complex media were added.

Death curves were constructed for *S. pyogenes* in the presence of three different concentrations of the raw whey fraction in which the thermogenic stimulatory property had not been activated.

RESULTS

Demonstration of the effect of raw whey on different bacteria. Growth curves show the sterilizing effect of raw whey on the growth of *S. pyogenes* (Figure 1).

The extended lag periods in the growth of *S. agalactiae* and *M. pyogenes*

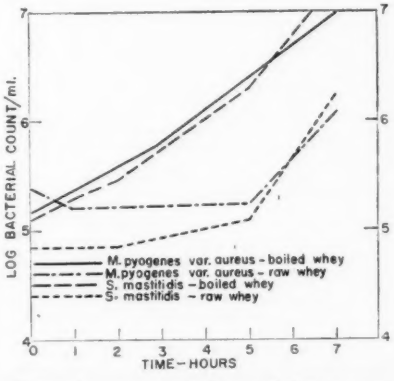
LOG BACTERIAL COUNT/ml.
M. pyogenes var. aureus - boiled whey
M. pyogenes var. aureus - raw whey
S. mastitidis - boiled whey
S. mastitidis - raw whey
TIME-HOURS

FIG. 2. The growth of *Streptococcus agalactiae* and *Micrococcus pyogenes* var. *aureus* in raw whey and in whey heated at 100° C. for five minutes.

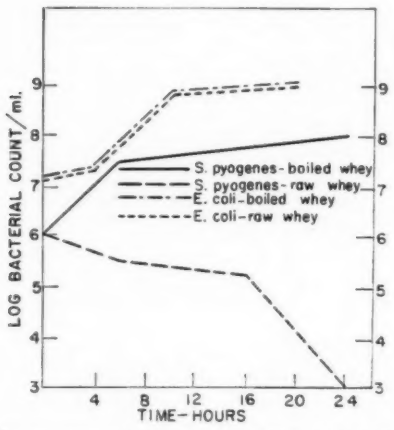

FIG. 3. The growth of *Streptococcus pyogenes* C203S and *Escherichia coli* in raw whey and in whey heated at 100° C. for five minutes.

var. *aureus* were observed in raw whey (Figure 2). Slopes of the logarithmic part of the curves in heated and raw whey were similar.

Growth of *E. coli* was not affected by the heating of raw whey. In marked contrast, the growth of *S. pyogenes* was significantly increased when whey was heated and used as a medium (Figure 3). *S. pyogenes* grew well (turbidity) in raw whey in which the thermogenic stimulatory property had not been activated, when heat infusion broth or yeast extract broth (20% by volume) was added to this raw whey.

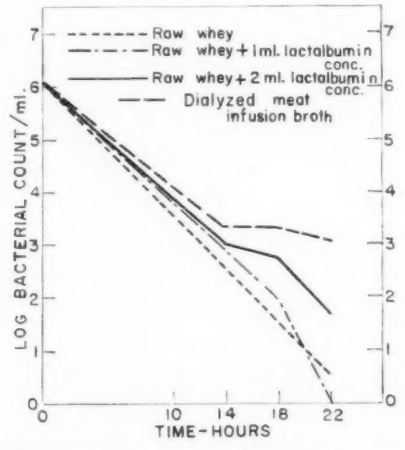
LOG BACTERIAL COUNT/ml.
TIME-HOURS
Raw whey
Raw whey + 1 ml. lactalbumin conc.
Raw whey + 2 ml. lactalbumin conc.
Dialyzed meat infusion broth

FIG. 4. The fate of *Streptococcus pyogenes* C203S in the presence of three different concentrations of unheated lactalbumin and in dialyzed meat infusion broth. One ml. of concentrated lactalbumin is equal to the content of the original whey. Meat infusion broth control showed normal growth (turbidity) in 22 hours at 37° C.

Microscopic examination of stained slides from all test cultures showed no evidence of clumping of bacteria, effectively ruling out an agglutinin as a cause of the decreased counts observed.

Death curves showing the fate of *S. pyogenes* in raw whey, raw whey supplemented with unheated lactalbumin concentrate, and dialyzed meat infusion broth—a nutritionally deficient medium—were similar (Figure 4), lending support to a nutritional deficiency concept. These curves were prepared to show that higher concentrations of unheated lactalbumin did not sterilize *S. pyogenes* in less time than the amount normally present in milk or whey. Since increasing concentrations of germicides usually attain given results in a shorter time interval, this experiment is taken as further evidence in favor of a nutritional deficiency concept.

Effects of the chemical components of a defined medium and of selected reducing agents on the growth of S. pyogenes. The chemical components of the defined medium (Table 1) failed to prevent sterilization of *S. pyogenes* C203S in raw whey when added singly and collectively in optimum concentration. Thus, after 24 hours, the plating of 1 ml. of the culture failed to produce any colonies, although one million cells per ml. of medium was originally added as inoculum.

Cultivation of this microorganism in raw whey medium, containing added reducing agents (Table 2), and also inoculated with one million bacteria per milliliter, resulted in growth when the same dilution was plated. The same cultures to which the reducing agents were added, plated at a dilution of one million, showed no growth. As the inoculum of *S. pyogenes* was between one and two million per milliliter, the results indicate that the lethal effect of raw whey for this microrganism was eliminated by the added reducing agents, but that the added reducing agents did not allow *S. pyogenes* to multiply.

Effect of heating whey and whey fractions on the growth of S. pyogenes. Effect of heating whey and whey fractions on the growth of *S. pyogenes* is shown (Table 3). Growth was observed only in instances in which free sulfhydryl groups (positive nitroprusside test) could be demonstrated. Free sulfhydryl groups were never found in raw whey or in whey heated insufficiently to activate the thermogenic stimulatory property. A five-minute holding period at 80° C., which activates the thermogenic stimulatory property, always produced free sulfhydryl groups.

Boiled whey contained free sulfhydryl groups, which gradually disappeared as the whey aged. The thermogenic stimulatory property was activated by boiling, but no growth could be demonstrated in aged whey in which free sulfhydryl groups were not evident. Reheating this whey failed to produce free sulfhydryl groups or to allow the growth of *S. pyogenes*.

The lactoglobulin and lactalbumin preparation for this study originally contained no free sulfhydryl groups. Heat-treating these preparations produced free sulfhydryl groups only in the lactalbumin preparation. When this heated preparation was introduced in raw whey, the whey supported growth, although this growth was not maximum.

TABLE 3

Effect of heating raw whey and albumin solutions on the nitroprusside test and the growth of Streptococcus pyogenes

Preparation	Intensity of nitroprusside reaction (free −SH groups)	Growth of *S. pyogenes*
		(*per ml.*)
Fresh, raw, sterile whey	−	Sterile
Fresh, sterile whey heated to 80° C.	−	Sterile
Fresh, sterile whey 5 min. at 80° C.	+	120,000,000
Fresh, sterile whey 10 min. at 80° C.	++	141,000,000
Aged, sterile, heated whey	−	1,000,000
Aged, sterile, heated whey reheated 5 min. at 80° C.	−	1,110,000
Lactoglobulin fraction in raw whey	−	Sterile
Lactoglobulin fraction 5 min. at 80° C. in raw whey	−	100,000
Lactalbumin fraction in raw whey	−	10,000
Lactalbumin fraction 5 min. at 80° C. in raw whey	+++	20,000,000
Pervaporated, sterile raw whey	−	Sterile
Pervaporated, sterile, raw whey 5 min. at 80° C.	+++	60,000,000
Sterile ovalbumin, 20 mg/ml added to raw whey	−	1,000,000
Sterile ovalbumin, 20 mg/ml 5 min. at 80° C. in raw whey	++++	15,000,000

Initial inoculum of *S. pyogenes* CS03S = 1,000,000/ml.
− to ++++ = intensity of nitroprusside reaction.

Results (presented in Figure 5) show that as the free sulfhydryl groups decreased in whey heated for extended periods, growth of *S. pyogenes* also decreased.

Two different wheys heated to 80° C. for short periods of time (Tables 4 and 5) show that the presence of free sulfhydryl groups invariably coincides with

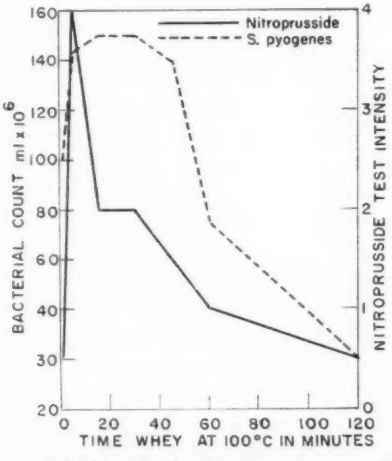

FIG. 5. The maximum population of *Streptococcus pyogenes* C203S in whey heated at 100° C. for various periods of time and the intensity of the nitroprusside reaction (free sulfhydryl groups) in whey resulting from similar heat treatments. Sterile raw whey with no free sulfhydryl groups.

TABLE 4

Effect of heating fresh, sterile, raw whey to 80° C. on the nitroprusside test and the growth of Streptococcus pyogenes

Sample heated	Nitroprusside (free −SH groups)	Final count/ml.
(*min.*)		
Unheated	−	Sterile
0	−	Sterile
1	−	Sterile
2	−	Sterile
3	+ (?)	9,000,000
4	+	64,000,000
5	++	41,000,000

Initial inoculum of *S. pyogenes* = 1,000,000/ml.
− to ++++ = intensity of nitroprusside reaction.

TABLE 5

Effect of heating sterile, aged, raw whey to 80° C. on the nitroprusside test and the growth of Streptococcus pyogenes

Sample heated	Nitroprusside (free −SH groups)	Final count/ml.
(*min.*)		
Unheated	−	4,000,000
0	−	34,000,000
1	−	30,000,000
2	+ (?)	67,000,000
3	+	131,000,000
4	+++	211,000,000
5	+++	151,000,000

Initial inoculum of *S. pyogenes* = 5,000,000/ml.
− to ++++ = intensity of nitroprusside reaction.

the activation of the thermogenic stimulatory property. These results are typical of four experiments.

Effect of cysteine and p*-chloro-mercuribenzoate treatment of heated lactalbumin fractions.* Cysteine in concentrations of 10^{-6} molar was ineffective in promoting growth of *S. pyogenes* in fresh and aged raw whey. At higher concentrations (10^{-4} molar) cysteine promoted the growth of *S. pyogenes* only in aged whey containing denatured protein as a result of heating.

Results of the addition of lactalbumin fractions, treated with various chemicals, to fresh, sterile, raw whey on the growth of *S. pyogenes* are shown (Table 6). Addition of *p*-chloro-mercuribenzoate (10^{-6} molar) to meat infusion broth prevented growth (turbidity) of *S. pyogenes*, and the addition of cysteine (3×10^{-6} molar) reversed this inhibition.

DISCUSSION

Results of growing microorganisms with different nutritional requirements in raw whey indicate clearly that *S. pyogenes* is sterilized but that *E. coli* is unaffected. *S. agalactiae* and *M. pyogenes* var. *aureus* apparently grew normally in raw whey after an extended lag period. These two microorganisms require

TABLE 6

Effect of the addition of various lactalbumin preparations to fresh, sterile, raw whey on the growth of Streptococcus pyogenes

Preparation	Final count/ml.
Unheated lactalbumin	1,000
Heated lactalbumin, 80° C., 5 min.	4,000,000
Heated lactalbumin, 80° C., 5 min. + excess *p*-chloro-mercuribenzoate and dialyzed	Sterile
Heated lactalbumin, 80° C., 5 min. + excess *p*-chloro-mercuribenzoate, dialyzed, + excess cysteine and dialyzed	40,000,000 [a]

Initial inoculum of *S. pyogenes* = 1,000,000/ml.

[a] No hemolysis evident, but selected colonies, after subculture in meat infusion broth, showed normal hemolysis. All components were sterile when tested.

more complex media for growth than *E. coli*, but less complex media than *S. pyogenes*. Although initially unable to multiply, they presumably can adapt (*15*) and grow well.

These results suggest that microorganisms, as a whole, might well be classified as sterilized, inhibited, or uninhibited, when subcultured in raw whey. *S. pyogenes* is the only microorganism known to be sterilized in this medium. Some unpublished experiments (by the present authors) indicate that *Sarcina lutea* and *Bacillus subtilis* may be classed in the inhibited group with *S. agalactiae* and *M. pyogenes* var. *aureus*. Past investigations indicate that *Aerobacter aerogenes*, *Salmonella typhosa*, *Streptococcus lactis*, and *Streptococcus cremoris* may also belong in this group. *Proteus vulgaris* and *Serratia marcescens* are generally uninhibited by the raw whey and belong in the class typified by the strain of *E. coli* used in this investigation. It is interesting to note that, generally, the three classes proposed are directly related to the complexity of the media required for growth, the sterilized class requiring the more complex media.

Ability of meat infusion broth and yeast extract broth to negate the effect of raw whey for *S. pyogenes*, and the similarity of death curves for this microorganism in raw whey and in nutritionally deficient medium (observations from this study), point to a nutritional deficiency as a significant factor in the inability of this microorganism to grow when subcultured in raw whey.

Experiments with reducing agents indicate that these compounds eliminate the lethal effect of the raw whey, but do not allow the growth of *S. pyogenes* in unheated whey, an observation dependent upon a quantitative, rather than a qualitative, determination of growth. Some of the reducing agents listed (Table 2) do not contain sulfur, but their effect in the raw whey was similar to that of the sulfur-containing reducing agents observed here and in earlier studies (*2*, *23*).

Occasionally in this study, *S. pyogenes* was not sterilized within 48 hours after exposure to the unheated whey, although the plate count diminished 95%. This usually occurred in aged whey and with a bacterial culture older than 18 hours.

That growth of *S. pyogenes* did not take place in raw whey until the heat treatment equalled that necessary for the liberation of free sulfhydryl groups,

is well illustrated in this work. The thermogenic stimulatory property of raw milk and whey has been shown to reside in the lactalbumin fraction of whey. This fraction is a source of free sulfhydryl groups in heated milk and whey, and is composed primarily of β-lactoglobulin (*11*).

P-chloro-mercuribenzoate, generally considered the most specific reagent for the binding of free sulfhydryl groups, prevented the growth of *S. pyogenes* in meat infusion broth and heated whey in which the thermogenic stimulatory property had been activated. Heated lactalbumin preparations treated with this reagent, with excess reagent dialyzed out, prevented the growth of *S. pyogenes* which resulted from the addition of heated, untreated lactalbumin to raw whey. In all cases this inhibition of growth was reversed by cysteine, indicating that free sulfhydryl groups are necessary for the growth of this microorganism. However, cysteine alone did not allow the multiplication of *S. pyogenes* in raw whey. The decreased growth of *S. pyogenes*, in whey, heated for extended periods of time, and in which free sulfhydryl groups decreased and eventually disappeared, also demonstrates the essential nature of these groups for growth of this microorganism.

In view of the conclusions of Slade and Slamp (*21*), it seems reasonable to assume that the heating of whey, which activates the thermogenic stimulatory property and liberates free sulfhydryl groups, also produces denatured albumin, which is required for the growth of *S. pyogenes*. The free sulfhydryl groups themselves are an indication of this denaturation, a view compatible with the results of other investigations (*4*, *6*, *7*, *11*, *19*, *20*).

It is suggested here that some microorganisms are inhibited or die out in the unheated milk or whey, largely because of a nutritional deficiency. This nutritional deficiency may be corrected by heating the milk or whey sufficiently to produce free sulfhydryl groups and to denature lactalbumin.

Investigation of heat-denatured lactalbumin preparations for streptogenin-like activity should prove interesting, since this presumed peptide is required for the growth of *S. pyogenes* and known to be stimulatory for many other microorganisms (*22*).

SUMMARY

S. pyogenes was an excellent microorganism for the detection of the germicidal property of milk or whey, which apparently was not due to an agglutinin or to lysis.

The growing of microorganisms with simple, intermediate, and complex media requirements, in raw whey, suggests that they may be classified as sterilized, inhibited, and uninhibited as a result of such exposure.

Reducing agents such as glutathione, mercaptoacetic acid, stannous chloride, sulfanilamide, sodium sulfite, thiamine, ascorbic acid, and hydroxylamine, in the concentrations used, eliminated the lethal effect of unheated whey for *S. pyogenes* but did not permit growth of this microorganism in unheated whey.

Addition of meat infusion and yeast extract broths allowed the growth of *S. pyogenes* in unheated whey, as did albumin preparations heated sufficiently to demonstrate the presence of free sulfhydryl groups.

The minimum heat treatment required to permit the growth of *S. pyogenes* in whey is also the minimum required for the detection of free sulfhydryl groups in milk or whey. The decrease in free sulfhydryl groups in whey, upon prolonged heating, coincides with the decreased growth of *S. pyogenes* in whey treated in a similar manner.

Experiments with *p*-chloro-mercuribenzoate and cysteine suggest that a deficiency of free sulfhydryl groups and/or denatured albumin in raw milk and whey is a major cause of the lethal effect of this medium for *S. pyogenes*.

ACKNOWLEDGMENTS

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SEDIMENTATION OF MILK PROTEINS FROM HEATED MILK

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The supernatant nitrogen in milk decreased immediately after heating, but rapid shifts in equilibria restored it during 24 hours of cold storage. Radioactive analyses for S^{35} showed that more than half of the whey proteins were in the gels of skimmilk heated to 203° F. *Editor.*

Ramsdell and Whittier *(6)* reported that heating milk for ten minutes at 212° F. produces no measurable aggregation or coagulation of the whey proteins. These results were based on centrifugation experiments in which the supernatant liquids were analyzed for acid-coagulable nitrogen. Edmondson and Tarassuk *(1)* obtained similar analytical results, but pointed out that some criteria were needed for differentiating casein from denatured whey protein before such a conclusion could be drawn from these observations. Such confirmation would appear particularly desirable in view of contradictory observations. For example, Patton and Josephson *(4)* found evidence of an association between the whey proteins and casein based on analyses for sulfhydryl groups. McGugan *et al. (3)* showed that interaction occurs between alpha-casein and beta-lactoglobulin in simplified systems.

Raw skimmilk contains an appreciable amount of soluble casein *(8)* which, under the influence of heat, may become centrifugable. However, any increase in centrifugable nitrogen beyond 78% of the total nitrogen indicates that denatured whey proteins either are sedimenting separately or are being carried down with the casein. In considering this approach, Pyne *(5)* and Hilgeman and Jenness *(2)* both observed a rapid change in soluble calcium and phosphorus immediately following the heat treatment of milk. Similar changes in centrifugable nitrogen might occur. Therefore, a study was made of the changes in centrifugable nitrogen as a function of time following several heat treatments. In addition, S^{35}-labeled whey proteins have been used to confirm the conclusion that heat treatment makes the whey proteins centrifugable.

EXPERIMENTAL PROCEDURE

In the first experiment, portions of skimmilk were heated in a tubular heater for one minute at 160, 220, or 280° F. and immediately chilled to 36° F. Samples of each milk, plus the raw skimmilk control, were centrifuged at intervals over a 46-hour period in the K rotor of the Spinco Model E Ultracentrifuge. Centrifugation was carried out under refrigeration for two hours,

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using an average centrifugal force of 113,000 times gravity. Under these conditions, essentially all of the centrifugable casein will be removed. However, analyses of the supernatant liquids from the raw skimmilk revealed variations related to the actual temperature of centrifugation, which varied in different runs between 40° and 50° F. Therefore, the analyses of the serums from the heated samples of skimmilk have in each case been referred to the corresponding values obtained with the unheated sample.

For the second experiment, S^{35}-labeled milk was obtained from Cornell University. After separating, the skimmilk was adjusted to pH 4.6 with dilute hydrochloric acid and the casein was removed. The whey was dialyzed against three changes of a neutral solution containing 15% sodium caseinate. Following a further dialysis against distilled water, the solution of whey proteins was freeze-dried. The dried product contained 53% protein. A concentrated solution of this powder was prepared and again dialyzed against distilled water to remove residual lactose and salts. It was then centrifuged for one and three-quarter hours at 110,000 times gravity, to remove any aggregates which might have been present. Twelve ml. of the clarified solution were added to skimmilk to give a final volume of 250 ml. Thus, the final concentration of whey proteins in this milk was approximately double that found in ordinary skimmilk. A 100-ml. portion was heated in a glass vessel for 15 minutes at 203° F. The heated sample was immediately cooled and both the raw and heated milks were centrifuged for one and three-quarter hours at 110,000 times gravity. The following day, samples were centrifuged under such conditions that roughly 25%, 50%, and 75% of the casein were removed. Supernatants and gels were analyzed for nitrogen and radioactive sulfur.

RESULTS AND DISCUSSION

Results of the first experiment are presented (Figure 1). The supernatant nitrogens in each of the heat-treated samples are plotted as per cent of those found in the raw skimmilk control. Immediately following the heat treatments, all samples show a marked decrease in protein content. The decrease is roughly proportional to the heat treatment in two cases. The analytical data suggest that some protein breakdown may have occurred in the sample heated to 280° F. Since the treatment at 220° F. produced the maximum effect, calculations will be based on this sample. A reduction in supernatant nitrogen to 33% of that found in raw skimmilk supernatant corresponds, in this case, to the centrifugation of 92% of the total nitrogen. Thus, there can be no question that whey proteins are being centrifuged immediately after this heat treatment.

All three samples showed a rapid shift in supernatant nitrogen following the heat treatments. The maximum observed at ten hours was not evident in the analyses for calcium. Since the temperature of the rotor during this run was unusually low, the nitrogen values may indicate a preferential solubilization of beta-casein *(7)*. Results obtained at 46 hours are not included in Figure 1, since they reveal no significant change from those at 28 hours. In approximately 24 hours, each sample shows a reversion to what appears to be an

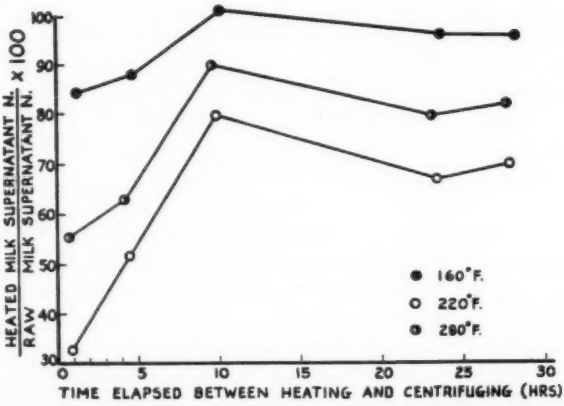

FIG. 1. Variation in supernatant nitrogen with extent of heating and time of analysis.

equilibrium value characteristic of the previous heat treatment. It is not clear at present whether this increase in soluble nitrogen is largely due to a re-establishment of the equilibrium between soluble and micellar casein or to a resolubilization of the whey proteins.

In the second experiment, using labeled whey proteins, it was found that the gels from raw skimmilk contained seven per cent of the radioactivity. This may be due largely to serum entrapped in the gels, although protein-protein interactions may occur even in raw skimmilk. With the heated skimmilk, 52% of the whey proteins was found in the gels. Partial centrifugation on the following day revealed little change in the amount of centrifugable whey proteins. It was also shown that the removal of these whey proteins was not due to the high centrifugal force that was used in the previous experiments. When 50% of the casein was centrifuged, approximately 62% of the denatured whey protein was removed.

SUMMARY

Milk is a very sensitive system and the most marked changes produced by heat treatments can be measured only when the time and temperature of fractionation are carefully controlled. Some of the observations which have previously been made and that appear contradictory may be attributed to differences in one or both of these factors. Immediately following the heat treatment of skimmilk, sedimentation reveals such a marked drop in supernatant nitrogen that it must be concluded whey proteins are being sedimented. As pointed out by Edmondson and Tarassuk *(1)*, the measurement of acid-coagulable nitrogen in the supernatant will not reveal this if the whey proteins are sedimenting uniformly with the casein.

During 24 hours of storage in the cold, rapid shifts in equilibria occur, which tend to restore the supernatant nitrogen to a degree dependent on the severity of the heat treatment. These results were confirmed, using skimmilk

containing added S^{35}-labeled whey proteins. Radioactive analyses demonstrated 52% of the whey proteins were present in the gels from a skimmilk which had received a strong heat treatment. In this case, considerable amounts of the denatured whey proteins remained centrifugable even after 24 hours' storage.

ACKNOWLEDGMENTS

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SPREADABILITY AND HARDNESS OF BUTTER.[1, 2] I. DEVELOPMENT OF AN INSTRUMENT FOR MEASURING SPREADABILITY[1]

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An instrument was developed more sensitive than consumers in detecting differences in the spreadability of butter. The results agreed closely with those obtained from consumer-preference studies. *Editor.*

The term "spreadability" has been used by consumers for many years to denote the ease with which butter may be spread on bread. Because the spreading action may be accomplished in various ways, however, a universally acceptable definition for spreadability has been lacking. This is unfortunate, because it has greatly retarded attempts to measure this property with objective physical methods. Like many other common terms, spreadability is not a single physical property, but rather a mixture of several. Scott Blair *(8)* defines spreadability as a psychological component in which a number of separate physical properties are concerned. He states that brittleness and hardness are the two principal factors involved.

Most investigators have studied the physical properties of butter by using instruments designed to measure hardness. The best-known methods of measuring hardness are the penetrometer, sectility, extrusion, and crushing-strength methods.

A reliable method of measuring spreadability as a separate entity has been sought by a number of investigators. Wilster *(11)* tested spreadability by putting butter directly on toast with an ordinary table-knife. Coulter and Combs *(1)* spread the butter on a glass plate by means of a knife fixed at a 45-degree angle. Lacy *(4)* attempted to standardize the organoleptic appraisal of spreadability by using a spreading blade which was attached to a trolley and was pulled over the butter by means of weights. Mohr and Haesing *(6)* modified Lacy's method by measuring the force required to pull a knife-blade across the surface of a block of butter. Griffiths *(3)* and Valentine and Sargent *(10)* tried to measure

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[1] Funds for partial support of these studies were made available by the Wuethrich Foundation, Greenwood, Wisconsin.

[2] Data presented are from a thesis submitted by Victor R. Huebner in partial fulfillment of the requirements for a Doctor of Philosophy degree.

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spreadability by determining the pressure required to force butter through a narrow orifice.

The temperature at which the spreadability of butter is measured must be controlled very carefully. Any butter can be made spreadable at a sufficiently high temperature, whereas even a very spreadable butter becomes brittle and hard at low temperatures. Reid and Arbuckle *(7)* made a study of temperature effects. They tempered butter samples for four hours at 40°, 50°, 60°, and 70° F., then spread them on bread. The butter was found to be very spreadable at 60° F., but higher temperatures caused salviness, and lower temperatures caused hardness and crumbliness. Dolby *(2)* has shown an almost direct relationship between temperature and hardness within the range of 43 to 64° F. Most investigators agree that butter should be spreadable at a temperature of 55 to 60° F. *(5)*.

DESCRIPTION OF THE INSTRUMENT [5]

The basic principles of Mohr and Haesing's *(6)* spreadability instrument were incorporated into the instrument used in this study. A schematic diagram is shown (Figure 1) and an actual photograph of the instrument is given (Figure 2). The butter-spreader portion of the knife has its blade beveled at a 45-degree

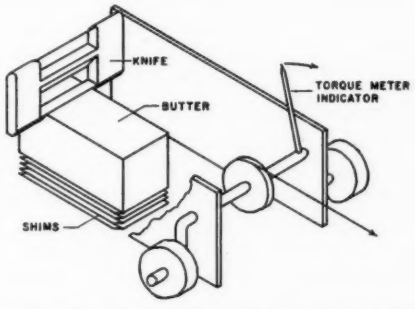

FIG. 1. Schematic diagram of the instrument used for measuring spreadability and hardness.

angle and the height of this beveled portion is $\frac{1}{16}$-inch. The top of the knife holds a 0.010-inch diameter stainless steel wire which is used to determine the hardness of butter. The carriage is designed so that the knife can be quickly inverted from the spreading position to the cutting position.

A block of butter 3 in. × $1\frac{1}{4}$ in. × $1\frac{1}{4}$ in. is placed in the stainless steel box. The upper surface of the butter is then leveled by moving the knife across it. A plastic shim is inserted beneath the butter to raise it the desired amount. A $\frac{1}{16}$-inch shim is used for a spreadability determination and a $\frac{1}{8}$-inch shim for a hardness determination. After the carriage is placed back of the butter, the

[5] An instrument using the same principles, but possessing certain engineering refinements, has been developed by Armour and Company. A replica of that instrument may be obtained from the Accurate Manufacturing Company, 945 King Avenue, Columbus, Ohio.

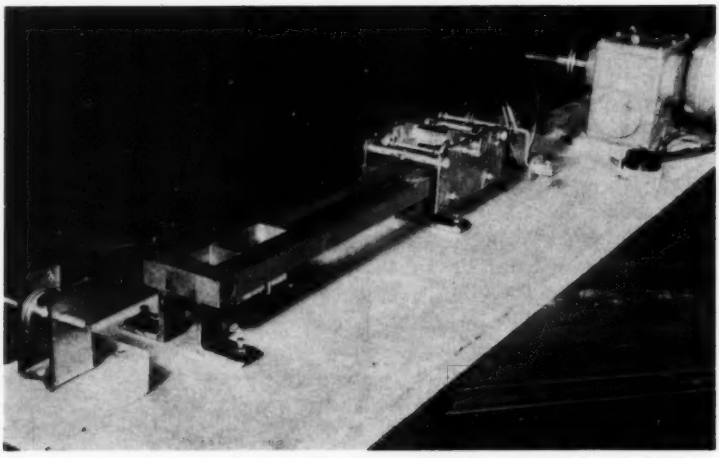

FIG. 2. Over-all view of the instrument used for measuring spreadability and hardness.

constant-speed motor pulls the knife through the butter at a rate of one centimeter per second. Resistance of butter to the knife is determined by the distance through which the torque meter indicator moves. This distance is measured on a plastic dial permanently attached to the carriage. The torque meter is calibrated by adding a series of known weights to the instrument and noting the readings when no butter is present. To make the device sufficiently sensitive, only a fraction of the resistance is actually measured by the torque meter. Before each test, known weights are added to the motor end of the apparatus and these weights are added to those registered on the torque meter.

Before testing, all butter samples are tempered for 48 hours in a room whose mean temperature is 55.5° F. The true temperature of this room fluctuates between 55.0 and 56.0° F. Four determinations for hardness and for spreadability are made on each sample in the 55.5° F. room. The standard deviation of the resistance to spreading values obtained on the same sample of butter is 4.3 gm., which gives confidence limits at the five-per cent level of significance of $\pm$ 8.6 gm.

SUBJECTIVE SPREADABILITY ASSESSMENTS

Consumer preference tests were made on the spreadability of eight different sets of samples in the University of Wisconsin dairy salesroom. An average of 50 persons graded the spreadability of each of the two samples being tested on any one particular day. Samples were all cut to the same size, held at 55° F., and spread on rye bread with table-knives. The subjective grades of poor, fair, good, and extra good were recorded by means of a numerical rating of 1, 2, 3, and 4, respectively. Average results of these consumer trials are compared (Table 1) with results obtained by the spreadability instrument.

Differences between samples used in Trials 3, 4, 5, 6, and 7 were statistically significant from the standpoint of both the instrument and the consumers. Sam-

TABLE 1

Relationship between consumers' rating of spreadability and resistance to spreading as measured by a rheological instrument

Trial No.	No. of consumers participating	Av. resistance to spreading[a]			Av. consumer rating[b]			L.S.D.[c]
		Sample A	Sample B	Mean difference	Sample A	Sample B	Mean difference	
		(gm.)	*(gm.)*	*(gm.)*				
1	38	868	816	52[d]	2.25	2.39	−0.14	0.38
2	52	829	816	13	2.74	2.32	+0.42[d]	0.34
3	45	1,206	832	374[d]	1.87	2.62	−0.75[d]	0.27
4	54	1,014	856	158[d]	2.20	2.72	−0.52[d]	0.35
5	50	938	276	662[d]	1.74	3.60	−1.86[d]	0.26
6	58	938	339	599[d]	1.83	3.64	−1.81[d]	0.19
7	53	938	601	337[d]	2.02	3.12	−1.10[d]	0.24
8	51	995	994	1	2.38	2.45	−0.07	0.29

[a] Average of four determinations.
[b] 1 = Poor; 2 = Fair; 3 = Good; 4 = Very Good.
[c] The smallest difference between consumer ratings which is significant at the 5% level.
[d] Significant at the 5% level of probability.

ples used in Trial 8 were not significantly different in either case. Only the spreadability instrument found a significant difference between samples used in Trial 1; whereas, only the consumers found a significant difference between those used in Trial 2. Samples used in Trial 2 were actually from the same churning, the only difference being that they were taken from different parts of the churn and that Sample B happened to be slightly leaky. (It is possible that the consumers discriminated against the leaky sample when making their spreadability assessments.)

Difference in consumer scores between each set of butter samples was compared with the amount of difference found by the spreadability instrument. These data were then analyzed with statistical procedures recommended by Snedecor *(9)*. It was calculated that a butter sample receiving an average score of one rating unit more than another would possess 314 gm. less resistance to spreading. Confidence limits at the five-per cent level of significance were found to be $\pm$ 32 gm. for this value. Because the spreadability instrument was found to have a sensitivity of $\pm$ 8.6 gm., it can be seen that it has an accuracy almost four times as great as that of the subjective assessments. Comparison between the differences obtained by the instrument, as compared to those by the consumers, had a correlation coefficient of −0.60, which was statistically highly significant ($P = <0.01$).

CORRELATION OF SPREADABILITY WITH HARDNESS

Spreadability values of 141 butter samples were compared with their respective hardness values. This comparison is illustrated (Figure 3). A highly significant correlation coefficient ($P = <0.01$) of 0.95 was obtained, indicating a close relationship between hardness and spreadability. The regression equation obtained by statistical analysis of these data was as follows:

$$\text{Resistance to spreading in gm.} = 90.9 + 2.63 \times \text{hardness in gm.}$$

The standard deviation from regression was found to be 43.4 gm., which gives

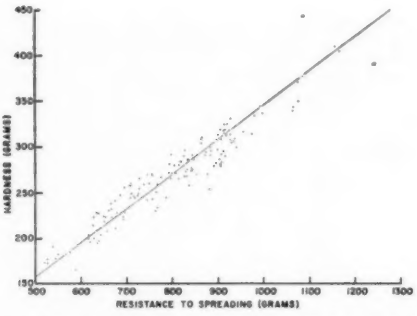

FIG. 3. Correlation between hardness and spreadability.

confidence limits at the five-per cent level of significance of $\pm$ 86.7 gm. Thus, while the most important factor affecting spreadability is hardness, certain other factors may exert minor influences.

SUMMARY

An instrument has been developed to measure the spreadability of butter. Although the instrument was much more sensitive than consumers in detecting differences in spreadability, results obtained with it were closely correlated with those obtained from consumer-preference studies. Hardness, as obtained with a sectility apparatus, has been found to be closely related to spreadability.

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SPREADABILITY AND HARDNESS OF BUTTER. II. SOME FACTORS AFFECTING SPREADABILITY AND HARDNESS[1,2]

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Temperature of the cream after pasteurization and that of the butter during storage and the printing procedure had the greatest influence on hardness and spreadability of butter. Storing cream at above 48° F. after pasteurization, and butter below 25° F., improved the body of butter. *Editor.*

With the advent of competitive spreads which emphasize superior spreadability, consumers of butter are becoming more spreadability-conscious. By eliminating the tempering of butter for several hours before using, its competitive position would undoubtedly be enhanced. The major purpose of this study was to find a practical means of improving spreadability of butter.

A number of factors have been shown to affect hardness and spreadability of butter. Coulter and Combs *(1)*, Haglund and Wode *(4)*, Lyons *(7)*, Storgards *(11)*, Wilster *et al. (12)*, and Wode *(13)* have consistently obtained much softer butter when the cream was held at higher temperatures prior to churning, even though the same final churning temperatures were used. Dolby *(2)* could find no consistent hardness differences in butter churned from cream held at 35°, 40°, or 45° F. In later work *(3)*, however, he obtained a much softer butter when he churned cream held at 60° F. rather than 48° F.

Previous investigations have shown that the length of time butter is stored has a great effect on its hardness. Data of Hunziker *(6)*, Mulder *(8)*, Prentice *(9)*, and Storgards *(11)* have shown an increase in hardness during storage. Their data indicate that the hardening process is quite rapid during the first week and then gradually slows down until very little increase in hardness is noticed upon further storage. Mulder *(8)* has attributed this hardening effect to a tendency of the crystals to grow together and interlock, thus forming a reticular structure. This process is usually termed "setting," or "hardening."

Recent investigations indicate that the setting or hardening process does not take place at an equal rate of speed for all storage temperatures. It was

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[2] Data presented are from a thesis submitted by Victor R. Huebner in partial fulfillment of the requirements for a Doctor of Philosophy degree.

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once commonly believed that the increase in firmness took place more rapidly at lower storage temperatures. As recently as 1934, Wode *(13)* stated that the greatest increase in hardness of butter occurred at low storage temperatures, but no data were given to support this claim. Conversely, Mulder *(8)* has shown recently that the setting process proceeds more rapidly at high storage temperatures.

Since the literature indicates some contradictory views with respect to effect of various handling procedures of cream and butter on spreadability and hardness, further experimental work appeared desirable. Effects of the milk fat composition, type and richness of the cream, cream cooling treatment, type of churn, working temperature, butter composition, butter storage conditions, and packaging procedures were studied. Variations in the cream cooling treatment, butter storage conditions, and packaging procedures were found to have the greatest effect on hardness and spreadability. Hence, these three factors will be the only ones discussed in this paper.

EXPERIMENTAL PROCEDURE

General. All cream was obtained from milk produced during the winter months by the same 30 herds of cows. All manufacturing operations were conducted in the University of Wisconsin dairy manufacturing laboratories. Because only one churning of butter could be made each day, many of the experiments were conducted over a period of two or three weeks. Iodine values of all butter samples were determined to verify the homogeneity of the milk fat within any one particular experiment. Iodine values of the fat in the experiments reported in the paper ranged from 31.28 to 38.82, with 55% of the 70 samples falling within a range of 32.50 to 34.50. Standard butter-making and handling procedures will be described in this section. Variations introduced into each experiment will be discussed under "Results and Discussion."

Cream treatment. Sweet cream with a fat content of 32 to 36% was pasteurized at 170° F. for 30 minutes in a round 300-gallon Damrow vapor-heated vat pasteurizer. This vat was also used to cool the cream to 38° F. in a period of two to two and one-half hours. The final stages of cooling were accomplished with chilled water. After cooling, the cream was held at 38° F. for 16 hours. It was tempered to 52° F. just before churning.

Churning. The 52° F. cream was pumped into an aluminum Vane churn with a capacity of 800 pounds of butter. Churning to granules approximately ¼-inch in diameter required 30 to 45 minutes. After draining the buttermilk, an equal amount of wash-water at 45° F. was added.

Working. After rotating the churn five to ten times, the wash-water was drained and the working process was begun. When the butter was approximately half-worked, it was standardized to 16.8% moisture and 2.0% salt. Working was then completed and the final temperature of the butter was taken. Final temperature of the butter was found consistently to be in a range of 56° F. to 57° F., except when temperature of working was purposely altered.

Storage. Samples of butter from each churning were wrapped in parch-

ment. They were stored at 36° F. for 14 days. All samples were tempered for 48 hours at 55.5° F. before being tested for spreadability and hardness.

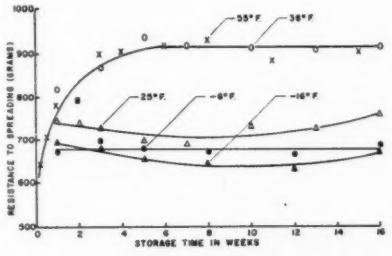
Automatic packaging. In those experiments in which butter was to be formed into one-pound bars, a portion of the churning was packed into stainless steel butter boxes which held 95 pounds of butter. These were cooled to an average temperature of 36° F. in a —16° F. freezing room in a period of eight hours. The temperature of the butter at this time ranged from 39° F. in the center of the box, to 32° F. at the outer surfaces of the butter. The butter was then stored in a 36° F. refrigerator until packaged. It was formed into one-pound bars in a Doering printer. Temperature of the butter as it emerged from the printer was 42 to 43° F.

Spreadability and hardness tests. Four determinations for hardness and for spreadability were made on each sample in a room held at 55.5°F. These tests were made with the apparatus described in an earlier paper *(5)*. Only the average spreadability and hardness values are given in this paper. Results were statistically analyzed by methods recommended by Snedecor *(10)*.

RESULTS AND DISCUSSION

Effect of butter storage conditions. Preliminary experiments indicated that butter stored at —6° F. retained its spreadability and softness characteristics during storage; whereas, butter stored at 36° F. rapidly became hard and unspreadable (all samples were tested at 55.5°F.). This phenomenon was further investigated. One-quarter pound portions of one churning were wrapped with individual aluminum foil wrappers. These packages of butter were then subdivided into five groups and stored in rooms at temperatures of —16°, —6°, 25°, 36°, and 55° F. Two packages of butter from each of the storage rooms were placed in the 55.5° F. testing room at suitable intervals during the 16-week storage period. After the customary 48-hour tempering period, this butter was tested for hardness and spreadability. Results of this experiment are shown (Figures 1 and 2).

It can be seen that resistance to spreading and hardness of the butter stored at 36° F. or 55° F. rapidly increased during the first few weeks of storage. Throughout the storage period, butter stored at 25° F. was softer and more



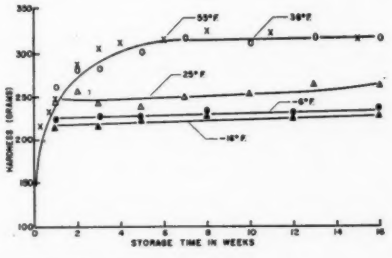
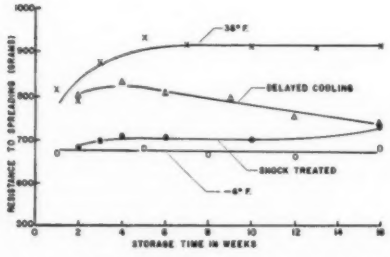


FIG. 1. Effect of storage time and temperature on spreadability.
FIG. 2. Effect of storage time and temperature on hardness.

spreadable than that stored at 36° F. or 55° F. Some further improvement was obtained by storing the butter at —6° F. or —16° F. These results indicate that the transition temperature at which the setting process is markedly halted lies somewhere between 36° F. and 25° F.

A portion of butter from the same churning was shock-treated by storing at —6° F. for seven days, at 36° F. for one day, and then at —6° F. for six days. This procedure was repeated for a total storage time of 16 weeks. Samples were taken after two, three, four, six, ten, and 16 weeks, and tested for hardness and spreadability. Another portion of butter was held at 36° F. for the first week and then stored at —6° F. for the remainder of the 16 weeks' storage period. Figure 3 shows the comparison between the spreadability of these specially treated samples, as compared with samples which were stored at a constant temperature of 36° F. or —6° F. Figure 4 shows this same comparison with respect to hardness.



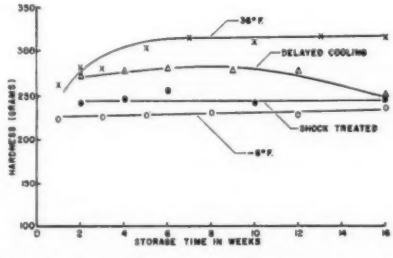


FIG. 3. Effect of delayed cooling and shock treating on spreadability.
FIG. 4. Effect of delayed cooling and shock treating on hardness.

Hardness and resistance to spreading values of the shock-treated samples were slightly greater than the respective values for the butter samples which had been stored constantly at —6° F. Hardness and resistance to spreading values of the shock-treated samples, however, were much lower than those of the samples which had been held at 36° F. throughout the storage period.

Resistance to spreading and hardness of butter which was held at 36° F. for one week before storing it at —6°F. gradually decreased after the fourth week of storage. After 16 weeks, hardness and resistance to spreading values of this butter were only slightly greater than those of the lot of butter which had been stored constantly at —6° F. These results indicate that the setting process may be reversed to some extent through the use of cold storage temperatures.

Effect of the temperature treatment of cream. Preliminary experiments indicated that cream cooled in a plate-type cooler produced harder and less spreadable butter than cream slowly cooled in a vat. Also, as the storage temperature of the cream after pasteurization and cooling was increased, butter churned from this cream was softer and more spreadable. These results were in agreement with results obtained by other investigators *(1, 3, 4, 7, 11, 12, 13)*.

It was desired to know whether a high cream storage temperature and a low butter storage temperature merely had the same effect on the structure of butter, or whether these treatments acted upon the butter independently of each other, thus creating an additive effect on improvement in spreadability.

Butter was produced from sweet cream which had been cooled to 38°, 48°, or 58° F. after pasteurization, and stored at these respective temperatures overnight. One portion of butter from each churning was stored at 36° F. for 14 days and another stored at —6°F. for 14 days. Results of the spreadability and hardness tests are given (Table 1).

TABLE 1

Effect of cream and butter holding temperatures on spreadability and hardness

Cream storage temperature	Churning No.	Hardness		Resistance to spreading	
		36° F.	—6° F.	36° F.	—6° F.
		(gm.)	*(gm.)*	*(gm.)*	*(gm.)*
	1	291	250	883	725
38° F.	2	308	261	899	750
	3	285	223	836	669
48° F.	4	266	207	811	662
	5	197	178	641	526
58° F.	6	200	183	641	599

These results indicate that hardness and spreadability are affected by both the cream holding temperature and the butter storage temperature. The softest and most spreadable butter was produced by cooling cream to 58° F. and holding the butter at —6° F. Results of the statistical analysis of these data are shown (Table 2).

TABLE 2

Analysis of variance on data from Table 1

Source of variance	Degrees of freedom	Hardness		Spreadability	
		Mean sq.	F value	Mean Sq.	F value
Butter storage temperature	1	19,927	54.5[a]	206,064	88.7[a]
Cream cooling treatment	2	31,817	86.9[a]	187,512	80.7[a]
Interaction	2	1,795	4.9	754	0.3
Butter treated alike	6	366		2,323	
Individual readings	36	4.9	0.01	18.4	0.008

[a] Statistically highly significant ($P = < 0.01$).

Effects of the butter storage temperature and the cream cooling treatment were both highly significant ($P = < 0.01$), but the interaction between these two treatments was nonsignificant. In other words, the two treatments did not interact with one another, but had cumulative effects on spreadability and hardness of butter.

Effect of printing. Various mechanical methods, most of which operate on a continuous basis, are used to form butter into one-pound, one-half pound, or one-quarter pound bars before it reaches consumers. This process is commonly termed "printing." The printer used in this experiment was a Doering, with a water-tempered auger.

Experiments were conducted to determine effects of printing on hardness and spreadability of butter. (Results of a typical experiment will be discussed in this section.) Portions of one churning were printed after five, 15, and 21 days of storage. Immediately after printing, a part of the printed butter was placed in the 55.5° F. tempering room. Other prints of butter were stored for 14 days at 36° F. and at —6° F. All samples of butter were tempered for 48 hours at 55.5° F. before testing them for spreadability and hardness. In addition, samples of the original unprinted butter were subjected to this same storage treatment immediately after churning. Results of this experiment are shown (Table 3). These are probably not directly applicable to all types of printers and all printing temperatures.

It can be seen that hardness and spreadability of the prints which were not stored after printing were approximately equal, regardless of length of storage time before printing. This effect is probably due to the vigorous re-

TABLE 3

Effect of printing on spreadability and hardness of butter [a]

Resistance to spreading [b]					
Control (not printed)		Printed butter			
Storage conditions after churning		Storage conditions after printing	Length of storage time before printing		
			5 days	15 days	21 days
	(gm.)		*(gm.)*	*(gm.)*	*(gm.)*
No storage	642	No storage	647	631	661
	637		650	638	650
14 days at —6° F.	605	14 days at —6° F.	607	611	647
	620		620	675	652
14 days at 36° F.	818	14 days at 36° F.	707	747	769
	798		715	756	772
Hardness [b]					
Control (not printed)		Printed butter			
Storage conditions after churning		Storage conditions after printing	Length of storage time before printing		
			5 days	15 days	21 days
	(gm.)		*(gm.)*	*(gm.)*	*(gm.)*
No storage	204	No storage	209	214	206
	195		208	217	208
14 days at —6° F.	216	14 days at —6° F.	193	184	202
	215		201	210	204
14 days at 36° F.	292	14 days at 36° F.	229	249	256
	286		239	255	247

[a] Individual values are averages of four determinations. Paired values represent replicate samples from the same lot of butter.

[b] Measured after standard tempering period of 48 hours at 55.5° F. following experimental storage period.

working which the augers of the printer gave the butter at the low printing temperature (36° F.) used in this experiment. Spreadability and hardness tests made on the unprinted butter at time of printing indicated that hardness and resistance to spreading values were considerably greater just before rather than just after printing. Hardness and resistance to spreading values returned to those of the freshly churned butter after the butter was printed. This indicates that the Doering printer is very effective in breaking the reticular structures in butter.

Values within each of the —6° F. storage columns were approximately equal, regardless of length of storage time before printing. Both resistance to spreading and hardness values of butter samples stored at —6° F. for 14 days after printing were nearly equal to those of the unstored butter samples. These results strengthen the idea previously expressed—that cold storage temperatures prevent the formation of a reticular structure in butter.

After 14 days of storage at 36° F., all the printed butters were softer and more spreadable than unprinted butter after a similar storage period. This indicates that the reworking which occurs during the printing operations causes a semi-permanent improvement to take place in the body of butter. This same phenomenon was noticed by Mulder *(8)* in his reworking experiments.

SUMMARY

Experiments were conducted to determine the effect of various butter-making procedures on hardness and spreadability of butter. Of the various treatments involved in converting the fat in milk into an acceptable table-spread, the cream storage temperature after pasteurization, the butter storage temperature, and the printing procedure were found to have the greatest influence on hardness and spreadability.

It was found that a high cream storage temperature (above 48° F.) after pasteurization and a low butter storage temperature (below 25° F.) would markedly improve the body characteristics of butter. These two treatments exerted independent effects on the structure of butter and did not interact with one another.

Experimental work on butter spreadability, in part reported upon in this paper, verified the findings of others on the influence of methods of cooling cream after pasteurization, holding temperatures prior to churning, and temperatures at which the granular butter is washed. The softest (most spreadable) butter was obtained when pasteurized cream was cooled slowly to 58° F. and held at that temperature until churning time (approximately 16 hours), using a butter wash-water temperature of less than 43° F., and storing the finished butter promptly at temperatures of —6° F.

It was also found that printing tends to rework butter. This reworking action, to a large extent, breaks the reticular structures that may have formed. It also prevents any new crystalline structures from becoming as unyielding as the previous ones within a 14-day storage period.

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DIGESTIBILITY AND BIOLOGICAL VALUE OF BACTERIAL CELLS

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Differences in biological value and digestibility exist between two nutritional types of bacteria. The digestibility of *Lactobacillus arabinosus* was greater than that of *Escherichia coli;* whereas, the reverse was true of their biological value. The type of nitrogen source in the growth medium did not alter the biological value of *Escherichia coli*. The biological value of *Escherichia coli* was increased when cellular preparations were supplemented with an amino acid mixture containing histidine, methionine, lysine, phenylalanine, and tryptophane. The omission of methionine from the supplement decreased the value. *Editor.*

The biological value of bacterial protein is of interest because of the role of the rumen microflora in utilization of nonprotein nitrogen *(3, 9, 15, 21)* and also because a portion of the nitrogen from protein degraded in the rumen *(8, 19)* is incorporated into bacterial protein.

Biological values ranging from 66 *(10)* to 88 *(16)* have been reported for mixed dried rumen bacteria, while other studies *(20)* have indicated that the biological value of rumen bacterial protein approximated that of casein. Investigators *(12, 13, 20)* have reported that rumen bacterial protein is deficient in methionine. Reed *et al. (20)* observed that rumen bacteria from sheep fed green forage contained more methionine than those from sheep receiving dry feeds. Tryptophan also has been suggested *(6)* as a limiting amino acid in microbial protein formed in sheep fed casein or gelatin.

Feeding trials suggest that rumen bacterial protein is deficient in methionine, but analysis of rumen ingesta from animals maintained on purified diets containing urea *(5, 14)* show that the ten essential amino acids are synthesized in large amounts in the rumen. In this connection, it has been reported that although some quantitative differences may occur between microorganisms, certain microbial proteins do not appear to differ materially from plant and animal protein, represented by wheat and beef liver *(23)*. Others *(2)* have found that the amino acid composition of casein, skimmilk powder, yeast protein, and *Lactobacillus fermenti* is similar.

The experiments described below were undertaken to determine whether fundamental differences in biological value and digestibility exist between pure cultures of two nutritional types of bacteria, and to assess some factors which may affect these properties.

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METHODS AND MATERIALS

Bacteriological. Escherichia coli, ATCC 8739, and *Lactobacillus arabinosus* ATCC 8041, were selected as test organisms, because they are nutritionally independent and dependent, respectively, in regard to requirements for preformed amino acids. Stock slant cultures of *E. coli* and stab cultures of *L. arabinosus* were maintained on Difco stock agar and were stored at 4° C. after initial incubation at 37° and 32.5° C., respectively.

Depending upon the nature of the experiment, *E. coli* was cultivated as a liquid culture in either a synthetic ammonium sulfate medium or in a complex tryptone-containing medium. The synthetic medium contained: 7 gm. K_2HPO_4, 3 gm. KH_2PO_4, 0.1 gm. $MgSO_4 \cdot 7H_2O$, 2 gm. $(NH_4)_2SO_4$, 1 gm. sodium citrate $\cdot$ $11H_2O$, and 5 gm. glucose, all dissolved in one liter of water and adjusted to pH 7.0. The complex medium contained: 10 gm. tryptone, 10 gm. yeast extract, 5 gm. K_2HPO_4, 5 gm. glucose, and 5 ml. of salt solution B per liter. Salt solution B contained 10 gm. $MgSO_4 \cdot 7H_2O$, 0.5 gm. NaCl, 0.5 gm. $FeSO_4 \cdot 7H_2O$, and 0.5 gm. $MnSO_4 \cdot 4H_2O$ dissolved in 250 ml. of water.

Large quantities of *E. coli* were grown with vigorous aeration at 37°C. in a carboy containing 15 liters of medium inoculated with one liter of an 18-hour culture. Vigorous aeration was attained by an apparatus which stirred at 172 r.p.m. and forced one to two volumes of filtered air per minute through the culture.

The cells were harvested in a Sharples Centrifuge when the culture was in the late log phase of growth, approximately six hours after inoculation. Growth was estimated turbidimetrically at desired intervals in an Evelyn Colorimeter equipped with a No. 660 filter. The centrifugate was twice washed with and centrifuged from cold 0.9% KCl. The packed cells were then either added to diets in this state, were lyophilized, or were sonic-oscillated for 30 minutes in a Raytheon 10 KC, 200-watt oscillator prior to lyophilization. The latter two cellular preparations were stored at 0° C. before use in diets.

The dried *E. coli* cells used in the experiment concerned with the supplementation of bacterial protein with specific amino acids were obtained through the cooperation of Dr. H. M. Tsuchiya, Northern Utilization Research Branch, Agricultural Research Service, United States Department of Agriculture, Peoria, Illinois. These cells were grown at 30° C. with vigorous aeration in 300 gallons of the ammonium sulfate medium in a deep fermentation type of apparatus. The cells were harvested by Sharples centrifugation after 16 hours of incubation, and were dried at 40° C. under vacuum.

Large quantities of *L. arabinosus* were grown without agitation or aeration at 32.5° C. for 18 hours, in carboys containing 17 liters of tryptone medium inoculated with 750 ml. of a 22-hour culture. This medium contained: 10 gm. tryptone, 5 gm. yeast extract, 5 gm. NaCl, 5 gm. K_2HPO_4, 5 gm. sodium citrate $\cdot$ $11H_2O$, 10 gm. glucose, 1 gm. "Tween 80" (sorbitan monooleate), 0.8 gm. $MgSO_4 \cdot 7H_2O$, 0.14 gm. $MnCl_2 \cdot 4H_2O$, 0.04 gm. $FeSO_4 \cdot 7H_2O$, and 100 γ of thiamin, all dissolved in one liter of water and adjusted to pH 7.0 *(7)*.

The cultures were harvested in the late log phase of growth and treated in the manner previously described for the processing of *E. coli cells.*

Sterilization of media and culture apparatus was accomplished by autoclaving at 121° C. for 20 minutes, or at 121° C. for one hour, when large volumes of media were contained in carboys.

Nutritional. The nutritional trials were conducted essentially in accordance with the procedure outlined by Mitchell *(17)*. Growing male albino rats weighing approximately 80 gm. were maintained on a controlled diet for approximately 30 days.

Endogenous urinary and metabolic fecal nitrogen values were calculated from data obtained during the last half of a 14-day preliminary period, during which the animals were fed a diet consisting of 24 parts of the basal ration (Table 1), plus one part of dried hexane-extracted, whole egg preparation. Total fecal and urinary nitrogen values were calculated from data obtained

TABLE 1

Composition of protein-free basal ration

Ingredient	Concentration
	(%)
Sucrose	12.0
Corn starch	37.0
Vitamin Mix A[a]	10.0
Vitavin Mix B[b]	1.0
Choline Chloride	0.4
P. H. Salt mixture[c]	4.0
Inositol	0.1
Cerelose	21.5
Wood floc	2.0

[a] Vitamin Mix A: 1 gm. tocopherol, 200,000 I. U. vitamin A, and 20,000 I. U. vitamin D dissolved in 100 gm. of corn oil.

[b] Vitamin Mix B: 25 mg. thiamine, 16 mg. riboflavin, 40 mg. pantothenate, 100 mg. nicotinic acid, 6 mg. pyridoxine, 0.6-mg. biotin, 2 mg. *p*-aminobenzoic acid, 0.02 mg. vitamin B_{12}, 1 mg. 2 methyl-1,4-naphthoquinone, and 4 mg. folic acid, all mixed with 9.8 gm. of glucose.

[c] Purchased from Nutritional Biochemical Corporation.

during the last half of a 14-day period, during which the rats were fed test diets consisting of the basal ration, supplemented with sufficient casein or bacterial preparation, to give the diets a 12% protein content calculated as $N \times 6.25$. The digestibility and biological value of the test proteins were calculated in the usual manner.

Diets containing dried egg, casein, or dried cell preparations were prepared about one week before use and were stored in sealed containers at 4° C. The nature of vegetative bacteria and the purpose of certain experiments precluded the preparation and storage of diets containing unprocessed bacterial cells in this manner. Therefore, diets containing freshly harvested and washed bacteria as a source of protein were prepared daily.

The experimental diets were fed to test groups consisting of four or five rats per group. Each rat, maintained at 28° C. in a metabolism cage, was fed a five-gm. portion of a selected diet once each day. The diets were weighed into porcelain feeding dishes designed to reduce feed scattering. Water, added in

amounts sufficient to render the diet semi-liquid, further minimized feed scattering and satisfied the water requirements of the rats. The diets were consumed within 90 minutes after presentation to the animals. Preliminary experiments demonstrated that there was no significant decrease in the viability of the unprocessed bacteria, when they were mixed with the diet and allowed to stand for 120 minutes.

Feces, collected separately from the urine, were removed daily and stored in sealed containers at 4° C. until analyzed. Urine was collected and accumulated throughout a trial in a Pyrex dish, in which 50 ml. of a solution containing one per cent sodium fluoride and two per cent cupric sulfate had been evaporated to dryness.

Chemical. The nitrogen content of test diets was determined on duplicate one-gm. samples by a modified Chibnall-Kjeldahl procedure *(3)*. The modification consisted of converting the procedure from micro to macro analysis by increasing the quantity of the reagents in accordance with the increase in sample size. Using urea or ammonium sulfate as standard, the recovery of nitrogen was 97%.

The urine in the collection dish was taken up in hot 0.1 *N* HS_2O_4 and filtered through glass wool into a 250-ml. volumetric flask. The dish was washed with three separate washings of hot 0.1 *N* H_2SO_4. The washings were filtered and combined with the acidified urine, which was then diluted to volume with distilled water. Duplicate 20-ml. aliquots were used for the determination of endogenous urinary nitrogen. Urinary nitrogen was determined on duplicate ten-ml. aliquots.

The entire fecal collection from each rat was digested separately in 500-ml. Kjeldahl flasks in the presence of concentrated H_2SO_4 and mercuric oxide as a catalyst. The digested feces were transferred quantitatively to 500-ml. volumetric flasks and diluted to volume with distilled water. Duplicate 200-ml. aliquots were used for the determination of fecal nitrogen by the Kjeldahl-Wilfarth-Gunning procedure *(1)*. Data from these experiments were subjected to an analysis of variance (F test).

RESULTS

The effect of drying on the digestibility and biological value of the bacteria was tested by feeding rats a basal diet, supplemented with either intact living cells, dried cells, or a dried sonic-oscillated preparation. For this purpose, a mass culture of *E. coli* was grown in ammonium sulfate medium, and harvested and processed as outlined in the previous section. Three diets, each containing 12% protein contributed by the respective bacterial preparations, and one diet containing casein at the same level, were fed to four groups of five rats each.

The data from this experiment are summarized (under Trial 1 in Table 2). The average digestibility of the untreated cells, the dried cells, and the dried, oscillated cell preparation was 80.8, 83.3, and 84.5, respectively. Biological values of 68.7, 66.2, and 70.2 were obtained for the respective preparations. An average digestibility of 99.6 and biological value of 81.7 were obtained from

TABLE 2

Digestibility and biological value of bacterial protein

Source of protein	N intake (*mg/day*)	Fecal N meta. total (*mg/day*)		Urinary N endo. total (*mg/day*)		True digestibility	Biological value
			Trial 1				
Untreated *E. coli*	107	7.2	28.6	18.6	45.7	80.8	68.7
Dried *E. coli*	100	7.8	24.8	18.2	46.2	83.3	66.2
Dried *E. coli* oscillate	95	7.6	22.3	20.1	44.0	84.5	70.2
Casein	90	7.4	7.8	18.8	35.2	99.6	81.7
			Trial 2				
Untreated *L. arabinosus*	89.9	7.7	21.7	13.1	49.2	84.3	52.3
Dried *L. arabinosus*	114.4	7.1	19.6	15.8	61.1	89.1	55.6
Casein	101.4	7.0	9.4	13.4	30.4	97.4	82.7
			Trial 3				
Dried *E. coli*	104.3	6.7	20.9	10.6	46.1	86.4	60.6
Dried *L. arabinosus*	108.9	6.7	17.6	11.1	62.2	90.0	47.8
Casein	87.8	7.6	8.5	11.7	30.6	99.0	78.3
			Trial 4				
NH_4-grown dried *E. coli*	99.9	6.6	20.0	10.6	47.0	86.6	61.1
Tryptone-grown dried *E. coli*	104.3	6.7	20.9	10.6	46.1	86.4	60.6
Casein	87.8	7.6	8.5	11.7	30.6	99.0	78.3

the control diet containing casein. Although the numerical difference in digestibility between untreated cells and dried preparations was not great, analysis of the data demonstrated that drying significantly ($P < 0.05$) increased the digestibility of the bacterial protein. The slight difference in digestibility between the dried and the dried, oscillated cells was not statistically significant. The differences in biological value between the three bacterial preparations were not significant at the five-per cent level. The digestibility and the biological value of casein were much higher ($P < 0.01$) than the values obtained from the diets containing bacterial protein.

Results similar to those obtained in the preceding trial were encountered in a later experiment, in which untreated and dried tryptone-grown cells of *L. arabinosus* were added to the basal diet as the source of protein. As shown (Trial 2 in Table 2), the mean digestibility of the untreated cells, the dried cells, and the casein was 84.3, 89.1, and 97.4, respectively. Biological values for the respective preparations were 52.3, 55.6, and 82.7. In this instance, the differences in digestibility between the untreated and the dried preparations were slightly lower than the values required for statistical significance.

Examination of the data from these two experiments suggests that the biological value of *E. coli* is greater than that of *L. arabinosus;* whereas, the digestibility of *L. arabinosus* is greater than that of *E. coli*. The biological value of either organism is lower than that of casein.

Although differences in digestibility and biological value between ammonium sulfate-grown *E. coli* protein and tryptone-grown *L. arabinosus* protein are evident from the foregoing data, it can be postulated that these differences are a reflection of the influence of the source of nitrogen in the growth medium on the

quality of synthesized bacterial protein. This was tested by feeding diets containing dried *E. coli* or dried *L. arabinosus*, both of which were grown in tryptone medium. Results of this experiment are summarized under Trial 3 in Table 2. As can be seen, the mean digestibility and the biological value of *E. coli* protein were 86.4 and 60.6, respectively, as compared to values of 90.0 and 47.8 for *L. arabinosus* protein, and 99.0 and 78.3 for casein. Analysis of these data demonstrated that the observed differences in digestibility and biological value between the three proteins were highly significant ($P < 0.01$). Thus, the higher biological value of *E. coli* protein as compared to *L. arabinosus* protein is not a function of the type of nitrogen in the growth medium, but rather is a characteristic of the species.

Additional evidence substantiating the hypothesis that the biological value of bacterial protein is a function of species or genus, rather than a function of the growth medium, was obtained by an experiment in which dried ammonium sulfate-grown and dried tryptone-grown *E. coli* cells were fed to rats. As shown under Trial 4 in Table 2, the digestibility and the biological values of the two bacterial preparations were nearly identical, and these values were significantly lower ($P < 0.01$) than the same values for casein.

In the preceding experiments, it was observed that the biological value of bacterial protein was lower than that of casein. The hypothesis that the lower biological value of bacterial protein may be the result of an amino acid deficiency in bacterial protein is strengthened by the examination of the amino acid composition of *E. coli (23)*, considered in terms of the amino acid requirements *(22)* of the growing male albino rat. A comparison of these values suggests that *E. coli* protein may be deficient in histidine, lysine, methionine, phenylalanine, and tryptophan. Therefore, an experiment was designed to test the effect of the addition of these amino acids on the biological value of *E. coli* protein.

A diet containing 99 mg. of dried *E. coli* nitrogen per five gm. was prepared and divided into seven equal portions, to which various combinations of the above listed amino acids were added. The concentrations of amino acids added were: 9.5 mg. L-histidine, 12.7 mg. L-lysine, 15.9 mg. L-methionine, 22.6 mg. L-phenylalanine, 3.4 mg. L-tryptophan and, when indicated, 7.0 mg. glycine. These concentrations of added amino acids, plus those present in the *E. coli* protein, were calculated to be slightly in excess of the rat requirement. A slight excess of each amino acid was added, to compensate for losses attributable to the indigestibility of *E. coli* protein.

The effect of the added amino acids was tested by the single omission technique, and results compared to the results obtained from a casein, and a glycine, control diet.

Data from this experiment are summarized (Table 3) and demonstrate that the biological value of *E. coli* protein was increased significantly ($P < 0.01$) by the addition of the five amino acids, as compared to the glycine control. The single omission of either histidine, lysine, phenylalanine, or tryptophan from the amino acid supplement did not affect the biological value of the cells. The omission of methionine from the amino acid supplement decreased the biological

TABLE 3

Effect of amino acid supplements on biological value of dried E. coli *protein*

Nitrogenous additions to basal ration	N intake (*mg/day*)	Fecal N meta. total (*mg/day*)		Urinary N endo. total (*mg/day*)		True digestibility	Biological value
Cells + A.A. mix. w/o Histidine	105.8	6.7	21.0	11.2	35.4	86.5	73.6
Cells + A.A. mix. w/o Lysine	106.3	6.8	20.8	12.2	38.5	86.7	71.5
Cells + A.A. mix. w/o Methionine	106.9	7.0	19.7	11.8	48.8	88.0	60.5
Cells + A.A. mix. w/o Phenylalanine	106.5	6.5	20.6	11.1	37.8	86.5	71.5
Cells + A.A. mix. w/o Tryptophan	108.0	6.5	20.9	10.6	36.3	86.7	72.6
Cells + A.A. mix. complete[a]	108.0	6.6	20.7	11.2	40.9	86.9	68.4
Cells + Glycine[b]	106.8	6.6	20.0	10.6	47.0	87.0	61.0
Casein	87.8	7.6	8.5	11.7	30.6	99.0	78.3

[a] The complete amino acid mixture contained: 9.5 mg. L-histidine, 12.7 mg. L-lysine, 19.5 mg. L-methionine, 22.6 mg. L-phenylalanine, and 3.4 mg. L-tryptophan.
[b] 7.0 mg. glycine added to cell control.

value to a level equivalent to that of the glycine control. These data show that, in relation to the rat requirements, a limiting amount of methionine is present in *E. coli* protein, and that the biological value of this protein is increased by addition of methionine. The data also show that the biological value of casein is significantly greater ($P < 0.01$) than that of methionine-supplemented *E. coli* protein.

DISCUSSION

The experimental data presented in this report show that the digestibility of the protein of *E. coli* and *L. arabinosus* cells is increased when the cells are dried. These results are consistent with data from similar experiments *(10)*, which demonstrated that the digestibility of yeast cells is increased by drying. Although the proteins of various species of bacteria may be similar to each other *(2)* and to other proteins *(23)* in regard to amino acid composition as determined microbiologically and/or chemically, the present data emphasize that significant differences exist in digestibility and biological value between casein and the proteins of *E. coli* and *L. arabinosus*. The observation that the digestibility and biological value of *E. coli* protein are independent of the source of nitrogen in the growth medium is in agreement with previous data *(23)*, which show that the qualitative and quantitative amino acid composition of a species of bacteria is a fixed and characteristic property of the cell.

The present data also confirm other observations *(20)* that microbial protein is deficient in methionine with respect to the requirements of rats for this amino acid. Under the conditions used to measure biological value, the maintenance requirements are met and growth is limited. Therefore, these results do not preclude the possibility that supplementation of the bacterial protein with lysine, histidine, phenylalanine, and tryptophan would not be beneficial under conditions

conducive to optimum growth. This possibility is strengthened by data *(18)* which show that the lysine requirement for growth is greater than that for maintenance. The similarities in the amino acid composition of *E. coli* and *L. arabinosus*, and the differences in biological value of these proteins, emphasize the fact that the amino acid analysis of a protein is not necessarily an index to its biological value, unless the protein is devoid of specific amino acids.

Since it has been demonstrated that the biological value of bacterial protein is independent of the nitrogen source used for growth, and that the biological value varies between species, it is suggested that those data which show differences in the biological value of rumen flora as affected by the ration fed, represent species shifts within the flora rather than changes in amino acid composition of individual species.

SUMMARY

Data from rat-feeding experiments have demonstrated that the digestibility of *E. coli* and *L. arabinosus* was increased when the cells of these organisms were dried before feeding. The digestibility of *L. arabinosus* was greater than that of *E. coli* when both were fed as dried preparations.

The biological value of *E. coli* was greater than that of *L. arabinosus*. The biological value of casein was greater than that of either bacterial preparation. Growth of *E. coli* on ammonium sulfate medium and on a tryptone medium did not alter the biological value of a dried *E. coli* preparation.

Addition of an amino acid supplement containing histidine, methionine, lysine, phenylalanine, and tryptophan to a dried *E. coli* preparation increased its biological value. The biological value of this preparation was decreased to a level equivalent to the glycine control, by the omission of methionine. The single omission of either histidine, lysine, phenylalanine, or tryptophan was without effect in this regard.

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SOME PHYSICAL PROPERTIES OF MILK. IV. MAXIMUM DENSITY OF MILK[1,2]

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Density determinations of milk-water mixtures were made, to determine if the temperature of maximum density is lower than that of water and if it is less as the milk in the mixture increases. It was found that as milk was diluted with water, the temperature of a maximum density of the mixture approached that of water in a linear way, but there were deviations at some points near the maxima. Linearity between temperature at maximum density and added water, and the large difference of temperatures for maximum density of milk and water, suggest a method for determining adulteration of milk. Variations of temperature of maximum density may indicate changes in the physical state of proteins. *Editor.*

The maximum density of water has long been recognized *(3)* as occurring near 4° C., but that of milk has not been reported uniformly. Davies *(2)* reported a maximum density of milk at −0.3° C. and Olson *(7)* reported one at −0.56° C. Neither writer cited references. Continuity of density at the freezing point has been contrasted with breaks or discontinuities of viscosity, and of electrical conductivity, at this temperature for several organic liquids *(5)*. Aqueous solutions usually have a maximum density *(8)*. Papers on theory of the liquid state *(1)*, supercooled liquids *(4)*, and spontaneous nucleation of water *(6)* also suggest that milk may have a maximum density. It was recently reported that milk does not have a maximum density above 0° C. *(9)*, but that there might be irregular changes near 4° C.

Investigations at this laboratory have now been extended, to study in greater detail the density of milk near 4° C. Densities of mixtures of water and milk were studied, to determine whether the maximum density shifts to a temperature lower than that of water, or whether this maximum grows less pronounced as the proportion of milk in the mixture increases.

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[1] Contribution No. 553, Department of Chemistry, and No. 251, Department of Dairy Husbandry, Kansas Agricultural Experiment Station, Manhattan.

[2] This paper contains part of the material presented by Thomas M. Medved, in a thesis for the Master of Science degree at Kansas State College, 1956.

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EXPERIMENTAL PROCEDURE

Density measurements were begun at 8° C., to start well above any irregularities due to the maximum density of water near 4° C. The lowest temperature reached, before freezing of the supercooled milk, was −6.75° C. For selected parts of this range, densities were determined at intervals of 0.25° C.

Milk used in these experiments was mixed-breed milk from the Kansas State College herd. Most samples which were selected for examination contained 3.6% fat and 12.7% total solids. Two of the samples used for studying effects of mixing milk with water contained 4.2% fat and 13.2% total solids. Each sample was commercially pasteurized at 143° F. for at least 30 minutes, homogenized at 2,000 p.s.i. in a Cherry-Burrell viscolizer, and cooled to 40° F. over a tubular cooler. Ages were measured from the time of cooling.

Densities were measured by hydrostatic weighing *(13)*. The bulb diameter was 28 mm. and its volume was approximately 100 ml. The bulb was weighed in water at several temperatures, and in air of known density. The coefficient of expansion thus found was used to calculate the exact bulb volume at any temperature. The bulb was connected through a hole in the balance floor to one arm of an analytical balance, by a fine nichrome wire. The sample was contained in a test tube approximately 35 × 250 mm. The diameter of the test tube was only slightly larger than the bulb, in order to produce gentle stirring of the sample when the balance arm was raised and lowered. It was essential to avoid scratching the test tube when samples were supercooled, because a scratch would cause the sample to freeze. Starting with 5° C. difference between the temperatures of sample and bath, changes in bulb weight, due to changes in the temperature of the bulb and the sample, were usually undetectable after five minutes and were assumed complete after 15 minutes. Starting with a difference of only 0.25° C., these times were two and five minutes. The immersed bulb acted as a damper, to make the balance swing slower than normal, but did not reduce the sensitivity below 10^{-4} gm. The precision, using a 100-bulb was, therefore, one in one million. The closeness in fit of points to smooth curves, including the straight lines in Figure 1, indicate that the reliability was equal to the precision.

A mechanical refrigerator, whose pump speed could be varied with cone pulleys, was adjusted to maintain the temperature of an alcohol-water bath slightly below each desired temperature. The bath, bulb, and sample were brought to the exact desired temperature with an electric heater and a slightly modified Sargent Model S Thermonitor. With these temperature controls, the bath temperature could be maintained with a precision of 0.001° C. through the temperature range of −6.75 to 8.00° C. Temperature settings were reliable to 0.01° C.

A 5-ohm platinum resistance thermometer, calibrated as described by Weber *(12)*, was used as a primary temperature reference. Beckman differential thermometers were then calibrated with the resistance thermometer, to serve as secondary standards for the Thermonitor settings.

Preliminary trials indicated that when milk was held at low refrigerator temperatures (3 to 4° C.), its temperature of maximum density decreased

markedly with age or time of storage during the first ten hours. After that, such changes as a result of aging were less pronounced. These findings seem consistent with findings of other workers studying changes in density *(10, 11)*. Therefore, densities of all samples reported were obtained after holding the samples for at least ten hours at approximately 3° C. after pasteurization, and then adjusting the samples to the desired temperature.

Density measurements on samples of milk diluted with water were started immediately after dilution of milk of ages 68 to 80 hours. Age effects under these conditions were disregarded.

RESULTS AND DISCUSSION

Figure 1 shows the results of a detailed study of the density of four milk samples through the temperature range of 3° C. to 5° C. Sample 3 was pasteurized, while Samples 1, 2, and 4 were both pasteurized and homogenized. Coefficients of correlation between temperatures and densities ranged between −0.997 and −0.999. These values substantiate the obvious conclusion that the density curves were smooth between 3° C. and 5° C.

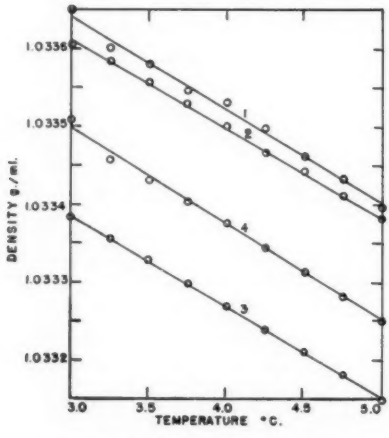

FIG. 1. Density *vs.* temperature.

Figure 2 shows the density over an interval of about 2° C., and the temperature of maximum density for a sample of milk and five dilutions with varying amounts of water. The ordinate scales for density are uniform for all curves, but the range for each curve differs. The interval of 0.00002 unit is shown for the dilution containing 20% milk. A single abscissa scale for temperatures applies to all curves.

In Curves A, C, D, E, and F containing, respectively, 100, 80, 60, 40, and 20% milk, the maxima are obvious, with considerably lower densities at both colder and warmer temperatures. The Dilution B, containing 90% milk, froze

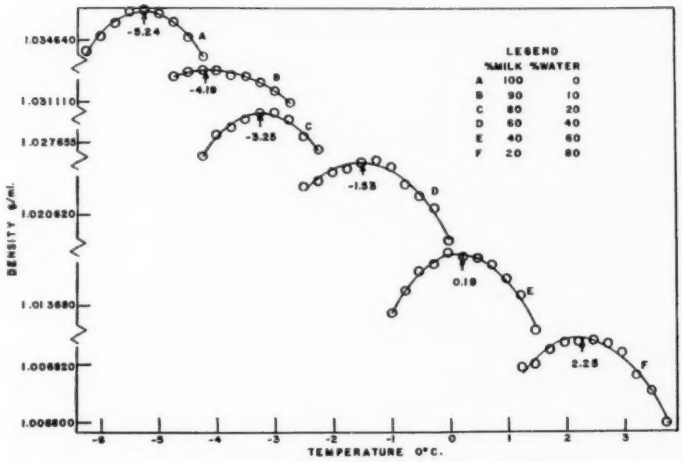

Fig 2. Temperature of maximum density of diluted milk.

before it became obvious that maximum density had been reached. The values for all five dilutions are consistent with values from similar dilutions of four other samples of milk. The maxima shown as arrows were obtained from parabolas, fitted by least squares to the data shown as small circles.

The deviations of some points near the maxima from their parabolas indicated a tendency toward secondary or twin maxima. This tendency was found in each of over 50 studies on various milk products. It may interest those studying maximum densities of other liquids. The parabolas gave a satisfactory fit within about 2° C. of the temperature of maximum density. Equations of higher than second degree were needed, to give even an approximate fit at temperatures farther from that of maximum density, and such equations did not fit so well as the parabolas in the ranges shown (Figure 2).

The relationship between the temperature of maximum density and the extent of dilution for the milk studied in Figure 2 is shown (Figure 3). As milk was diluted with water, the temperature of maximum density approached that of water in a linear manner. The regression line was fitted by least squares and indicates a temperature of maximum density of 3.99° C. for water. The coefficient of correlation was 0.999. This result is consistent with results from two other studies, except that ages at dilution were less well-controlled in those studies.

In addition to the data reported in Figures 2 and 3, maximum densities were studied on seven samples of milk, at ages ranging from one to 300 hours. Values for the temperature of maximum density ranged from −6.00° C. at age 12 hours, to −3.48° C. at 240 hours. At age ten hours after pasteurization, the average temperature of maximum density for the seven samples of milk was −5.20° C.

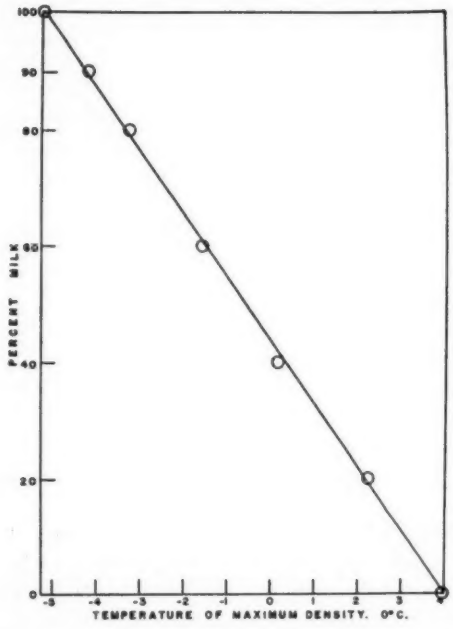

Fig. 3. Changes in maximum density of diluted milk.

The linearity of relationship between temperature of maximum density and percentage of added water in milk, and the relatively large difference of temperatures for maximum density of milk and water, suggest a possible method for determining the adulteration of milk with water. The differences between samples, and the changes with age in a given sample, are serious problems in developing such an application. Changes of the temperature of maximum density of milk, as well as changes in viscosity *(14, 15)*, may have greatest practical value as indicators of changes in the condition or state of protein particles in the milk. Optimum conditions or states of protein particles may well improve the protein stability of evaporated milk, may permit the use of larger amounts of milk in bread, may encourage the use of reconstituted dry milk in cottage cheese and as a beverage, and may contribute to improved flavor, texture, and other factors influencing consumer acceptance of many foods containing milk proteins.

CONCLUSIONS

A smooth curve for density *vs.* temperature of milk was found near 4° C.

As milk was diluted with water, the temperature of maximum density of the mixture approached that of water in a linear manner, with respect to percentage composition. The deviations of some points near the maxima from their parabolas indicated a tendency toward secondary or twin maxima.

Ten hours after pasteurization, the average temperature of maximum density for seven samples of milk was −5.20° C.

Several possible practical applications of changes in the density and viscosity of milk are suggested.

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EFFECTS OF PASTEURIZATION TEMPERATURES AND EXPOSURE TO LIGHT ON HOMOGENIZED MILK

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Homogenized milks, pasteurized at 163 to 183° for 17 seconds in 5° F. intervals, were held in clear-glass bottles at 40° F. in the dark and also under 20 ft-c of light for zero, one, two, and three days. Loss of ascorbic acid and intensity of oxidized flavor decreased with increased pasteurization temperatures. *Editor.*

Previous research has indicated the detrimental effect of artificial light in cold-storage rooms on the flavor and ascorbic acid retention in milk bottled in clear glass *(3)*. Doan *(1)* found that higher than normal pasteurization temperatures decreased the development of oxidized flavor and increased the level of ascorbic acid retention in homogenized milk exposed to sunlight. These findings are in agreement with tendencies noted in studies of the effects of artificial light on homogenized milk *(3)*.

The objective of this study was to determine the effect of various pasteurization temperatures on the development of oxidized flavor and the retention of ascorbic acid in homogenized milk, bottled in clear glass and exposed to artificial light.

EXPERIMENTAL PROCEDURE

Mixed-herd milk, as delivered every second day from the University dairy barn during December and January, and every third day during July and August, was used for the winter and summer trials, respectively. The difference in the delivery time of the winter and summer milk was due to processing difficulties beyond the control of the authors. The milk was clarified, homogenized at 2,000 p.s.i., and pasteurized by the high-temperature short-time method at 163, 168, 173, 178, 183, and 188° F. for 17 seconds. The milks were cooled to 36° F. by means of a plate-type cooler and bottled in half-pint clear-glass bottles. The bottles were sealed with Seal-Hood caps. Approximately two hours elapsed between the end of the last morning's milking and the completion of the bottling process.

Equal numbers of half-pint samples of the pasteurized milks were placed randomly in half-pint milk cases and stored at 40° F. in each of two milk-storage rooms containing, respectively, no light and a 100-watt incandescent light. A sufficient number of samples was prepared so that each milk was exposed to no light or to 20 ft-c of incandescent light for zero-, one-, two-, and three-day periods. The milk cases containing the samples to be exposed to light were

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placed on top of other cases, at such a level that the light source from above was at the desired intensity when measured by means of a light meter. The light intensity was measured at the top of each sample case and at a point level with the top of the milk bottles. The samples were removed at random, at the end of each storage period, and randomized prior to their analysis.

The criteria used to determine the effect of light on the bottled milk were ascorbic acid content and degree of oxidized flavor. Ascorbic acid expressed as milligrams per liter was determined by the method of Woessner *et al. (5)*. This method was modified so as to permit the use of a Klett-Summerson photoelectric colorimeter. Flavor scores from judgments of four individuals were totaled and used to indicate the degree of oxidized flavor. The intensity of oxidized flavor was expressed as follows: absent (0), slight (1), distinct (2), and strong (3). The character of the flavor reported in this study is referred to as oxidized. However, the flavor was not typical of an oxidized or activated flavor, but was possibly a blend of both types of flavor defects.

Ascorbic acid concentration and oxidized flavor score data on winter and summer milk samples exposed to light were analyzed independently. The analyses included variability among temperatures of pasteurization and days in storage, as well as the interactions of these two main effects. In addition, trends among temperatures of pasteurization and among days in storage were explored *(4)*. The total scores representing the degree of oxidized flavor were transformed into the square root of the total value plus 0.5, because of the preponderance of zero values *(4)*.

The data for the milk samples stored in the dark are included in the tables for comparison only. The ascorbic acid concentration and degree of oxidized flavor of the milk held in the dark were not analyzed, because of similarity of the data to those analyzed in a previous report *(3)*.

RESULTS

The effects of temperatures of pasteurization and days in cold storage during the winter and summer months, on ascorbic acid levels in homogenized milk exposed to no light and to 20 ft-c of light, are shown (Table 1).

The analysis of variance of the ascorbic acid levels in homogenized milk exposed to light indicated that changes in the temperature of pasteurization within the limits studied resulted in differences in the retention of ascorbic acid for the winter and summer milk ($P<0.001$ for each milk). The retention of ascorbic acid was proportional to increases in pasteurization temperatures for the winter and summer milk ($P<0.001$ for the linear trend for each milk).

A comparison of the ascorbic acid levels in the samples exposed to no light and light indicated the detrimental effect of artificial light on the ascorbic acid retention in milk bottled in clear glass, which is in agreement with a previous report *(3)*.

As was anticipated, a difference in the ascorbic acid content in both winter and summer milk stored under light for zero-, one-, two-, and three-day periods was observed ($P<0.001$ for each milk). The concentration of ascorbic acid

TABLE 1

Effect of temperatures of pasteurization on ascorbic acid levels in homogenized milk exposed to no light and 20 ft-c of light during the summer and winter months

Temperature of pasteurization	Light intensity	Days in storage			
		0	1	2	3
(°F.)	*(ft-c)*	*Ascorbic Acid (mg/liter)*			
		Summer milk [b]			
163	0	11.3[a]	8.6	6.7	3.2
168	0	11.7	9.4	8.4	4.5
173	0	11.3	10.6	8.8	7.9
178	0	11.6	10.9	9.2	9.1
183	0	11.3	10.8	10.3	9.8
188	0	11.0	10.9	10.6	10.3
163	20	11.0	6.9	4.2	2.0
168	20	11.2	8.3	6.5	2.4
173	20	10.8	9.2	7.3	3.6
178	20	10.9	9.9	8.5	6.5
183	20	10.9	10.3	8.6	7.2
188	20	11.3	10.3	9.4	7.7
		Winter milk [c]			
163	0	14.3	11.6	7.6	4.7
168	0	13.9	12.4	10.5	9.0
173	0	13.9	13.3	12.6	11.6
178	0	14.7	13.7	13.4	13.0
183	0	14.0	14.1	13.6	12.9
188	0	14.3	14.2	13.9	14.0
163	20	14.4	9.1	5.4	1.9
168	20	14.6	10.4	7.3	4.8
173	20	14.7	12.6	8.8	5.2
178	20	14.5	12.2	10.1	7.7
183	20	13.9	12.5	9.9	8.0
188	20	14.2	13.3	10.7	8.6

[a] Average for three replicates.

[b] Standard error for any value for summer milk exposed to light $\sqrt{\frac{EMS}{3}} = 0.48$.

[c] Standard error for any value for winter milk exposed to light $\sqrt{\frac{EMS}{3}} = 0.62$.

decreased in a uniform manner with time in storage ($P<0.001$ for each milk). The samples stored in the dark retained greater levels of ascorbic acid than those exposed to light, which is in agreement with previous findings *(3)*.

Effects of temperatures of pasteurization and days in cold storage during the winter and summer months on the degree of oxidized flavor ($\sqrt{\text{total score}+0.5}$), in homogenized milk exposed to no light and to 20 ft-c of light, are shown (Table 2).

The analysis of variance of the transformed flavor scores, representative of the development of oxidized flavor in homogenized milk exposed to light, revealed that changes in the temperature of pasteurization within the limits studied resulted in differences in the degree of oxidized flavor development for the winter and summer milk ($P<0.001$ for each milk). The degree of oxidized flavor development was inversely proportional to increases in pasteurization temperatures for the winter and summer milk ($P<0.001$ for the linear trend

TABLE 2

Effect of temperature of pasteurization on degree of oxidized flavor, in homogenized milk exposed to no light and 20 ft-c of light during the summer and winter months

Temperature of pasteurization	Light intensity	Days in storage			
		0	1	2	3
(°F.)	(ft-c)	(Degree of oxidized flavor)			
		Summer milk [b]			
163	0	0.7[a]	0.7	0.7	1.1
168	0	0.9	0.7	0.7	0.9
173	0	0.7	0.7	0.9	0.7
178	0	0.7	0.7	0.7	0.7
183	0	0.7	0.7	0.7	0.7
188	0	0.7	0.7	0.7	0.7
163	20	0.7	2.3	2.9	2.5
168	20	0.7	1.9	2.8	2.5
173	20	0.7	1.1	2.2	2.6
178	20	0.7	1.1	1.4	2.3
183	20	0.7	1.1	1.0	2.0
188	20	0.7	0.7	0.9	1.5
		Winter milk [c]			
163	0	0.7	0.7	0.7	0.9
168	0	0.7	0.7	1.2	1.0
173	0	0.7	0.9	0.7	0.7
178	0	0.7	0.7	0.7	0.9
183	0	0.7	0.7	0.9	0.7
188	0	0.7	0.7	0.7	0.7
163	20	0.7	1.9	2.8	3.0
168	20	0.7	1.9	2.9	3.0
173	20	0.7	1.3	2.5	3.1
178	20	0.7	1.5	2.3	2.8
183	20	0.7	0.9	1.8	2.1
188	20	0.7	0.9	1.4	1.3

[a] Average for three replicates of $\sqrt{\text{total score} + 0.5}$, to the nearest tenth.

[b] Standard error for any value for summer milk exposed to light $\sqrt{\frac{EMS}{3}} = 0.20$.

[c] Standard error for any value for winter milk exposed to light $\sqrt{\frac{EMS}{3}} = 0.19$.

for each milk).

A comparison of the degree of oxidized flavor in the samples exposed to no light and light indicated, as previously reported *(3)*, the detrimental effect of artificial light on the flavor of milk bottled in clear glass.

As was anticipated, a difference in the degree of oxidized flavor development in both winter and summer milk stored under light for zero-, one-, two-, and three-day periods was observed ($P<0.001$ for each milk). The degree of off-flavor development increased directly with days in storage ($P<0.001$ for each milk). The samples stored in the dark had less incidence of oxidized flavor than those exposed to light, which is in agreement with previous findings *(3)*.

DISCUSSION

The results indicate that increasing the temperature of pasteurization, within the limits studied for homogenized milk bottled in clear glass, increased ascorbic

acid retention and decreased susceptibility to oxidized flavor development. However, pasteurization temperatures of 178° F. and above resulted in a marked cooked flavor which did not disappear completely with storage. Exposure of homogenized milk to light is detrimental, but increasing the temperature of pasteurization, to a point where cooked flavor is not objectionable, will tend to counteract the effect of light.

A few practical dairy plant operators have believed that higher than normal temperatures of pasteurization would increase light-induced oxidized flavor development. This may be due to the higher temperatures of pasteurization, resulting in greater protein denaturation. The presence of this denatured protein may affect light absorption and contribute to the induction of off-flavors. Conversely, higher temperatures of pasteurization, particularly 168° F. and above, may result in greater production of sulfhydryls, which act as antioxidants and tend to prevent oxidized flavor development *(2)*. The decreases in oxidized flavor levels associated with increased temperatures of pasteurization (as shown in the data in Table 2), suggest that the sulfhydryl effect is greater than, and/or overcomes, the possible effect of greater protein denaturation.

SUMMARY

Samples of homogenized milk, pasteurized at 163, 168, 173, 178, 183, and 188° F., respectively, for 17 seconds during the winter and summer months, and bottled in clear glass, were stored at 40° F. in the dark and under 20 ft-c of incandescent light for zero, one, two, and three days.

The rate of loss of ascorbic acid and degree of oxidized flavor development decreased with increases in pasteurization temperatures for the winter and summer homogenized milk samples exposed to light.

ACKNOWLEDGMENT

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OUR INDUSTRY TODAY

Brief Reviews of Current Topics

Dairying in India[1]

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Milk is an important source of animal protein and fat in India, where a large majority of the people are vegetarians. Dairying in this country is in the developing stage. In spite of the great numbers of cattle, milk production and milk consumption are low. Milk and its products are relatively expensive for the average Indian.

Geography. The Republic of India, a subcontinent in southeast Asia, is situated entirely north of the equator, with its lowest tip at latitude 8°. It spreads over 1,700 miles from east to west and about 2,000 miles from north to south. It has a total land area of about 1.3 million square miles — about half the size of the United States. The total population is nearly 370 millions, or more than twice that of the United States.

Agriculture. India is primarily an agricultural country. The Government report *(7)* shows that although there has been a steady shift towards urbanization, only 17% of the population live in about 3,000 towns and cities, 83% live in more than 600,000 villages, and 500,000 of these villages have a population of less than 1,000 each. More than 250 million people, or 70%, depend directly on land, and about 30% are engaged in various industries, commerce, and other occupations.

The agriculture of India, with its wide range of soils and physical and climatological conditions, varies greatly. The climate for the greater part of the year is warm—in the northwest the average minimum and maximum temperatures for January are 47 and 74° F., respectively, and for May, the hottest month, 78 and 103° F. The dominant factor in Indian agriculture is the monsoon, which brings rain between June and October. The average annual rainfall for the country is 45 inches, but it is so irregular that many tracts of land are irrigated *(11)*.

Indian soils are generally deficient in organic matter. Although this problem could be partially solved by returning manure to the land, the problem has persisted because cattle dung is largely used for village fuel. Farming for the most part is primitive, with little capital invested in machinery or equipment. Farmers are mostly illiterate and because of their economic situation use few and simple implements. The plows are usually made of wood with iron or steel tips. Bullocks are used for plowing, raising water from wells for irrigation, threshing, and running simple, indigenous machinery. However, during the past few years great efforts have been made towards mechanizing and modernizing agriculture.

Dairying. Dairying in India is a much more integral part of agriculture than it is in the West. Although there are a few well-established government and military dairy farms, the average farmer owns from one to two milk animals and from two to three bullocks *(5)*. Since the male animals are used for motive power, they are considered the more valuable, and dairying is regarded as only a subsidiary occupation.

Compared to that in the Western World, the dairy industry in India is underdeveloped and backward. Nevertheless, approximately 1.5 billion dollars worth of milk and milk products are produced annually, and it may be considered one of the major industries.

Dairy cattle and milk production. India possesses more than 200 million cattle, comprising nearly one-third of the world's cattle population. The milk production depends upon approximately 46 million Zebu-type milk cows (*Bos indicus*) and 21 million buffaloes (*Bos bubalis*). There are also about 8 million milk-goats, but their milk production is commercially unimportant.

Although there exist some good milk-breeds, like Red Sindhi, Sahiwal, and Gir cows, and Murrah buffaloes, the large majority of the animals are extremely poor producers of milk. Ignorance and indifference on the part of the farmers have resulted in promiscuous breeding, which has given rise to a huge number of poor animals of nondescript breeds.

In India, 40 billion pounds of milk are produced annually from 67 million cows and buffaloes, compared to 124 billion pounds produced annually in this country from only 22 million cows. The average annual milk production per

[1] Article 7:57 of the Department of Dairy Technology, The Ohio State University.

animal in India is 800 lb., as compared to 5,500 lb. produced by the average American cow. For dairy purposes buffaloes are more important *(14)*. Though they constitute only 30% of the milk animals, they account for nearly 56% of the total milk production. Annually, an average buffalo yields 1,100 lb. of milk with from 6.5 to 7.5% fat, as against 413 lb. of milk with from 4.5 to 5% fat for the cow.

Due to poor farming methods, inadequate irrigation facilities, and lack of fertilizers, not enough animal feed is available for the large cattle population. Poverty, and backwardness in applying scientific methods, have encouraged improper management, breeding, and sanitation. Inadequate veterinarian help has contributed to the prevalence and multiplication of animal diseases. The situation is further complicated by the religious sentiment of the Hindus, which forbids the slaughtering of cows; because of this, low-yielding animals continue to consume food which could be utilized more economically by better producers.

A considerable number of milk animals are maintained (nearly 13.3 millions) within the city areas, where milk is produced for human consumption. Factors responsible for this are inadequate facilities for transporting milk from the country, quick spoilage due to the warm climate, and lure of better prices in the city. Some of the temples, religious people, and wealthy families maintain their own animals for a timely and unadulterated milk supply. Although the system provides fresh milk to the consumer, the situation has created unsanitary conditions in the city areas and has been a potential public health hazard.

Handling of milk. Since individual farmers own the cattle in small numbers, they obtain a limited quantity of milk, ranging between 4 to 10 lb. of milk at each milking. After retaining a part of their milk supply for domestic use, they sell the rest to a village shop or to a village milk collector. A small minority of farmers sell their milk directly to dairies in nearby cities or to cooperative milk societies. The collector receives the milk, measures it, and pays for it in cash. Every morning and evening, an average of from 60 to 80 lb. of milk is collected in brass, copper, or earthen vessels, and in some instances in cans, and transported immediately to a nearby town or city. It is transported as "headloads," on bicycles or in horse-, pony-, or donkey-driven carts. Motor transportation is employed in a few cases, in which the quantity of milk is substantial. In general, no testing of the milk is carried out. No efforts are made to refrigerate or pasteurize the milk. The milk is sold as raw milk from door to door or in milk shops, or is sold to another, larger collector or to a dairy in a nearby town or city for retailing. Khurody *(9)* has estimated that about 98% of the milk is distributed raw.

There are a few milk cooperative societies which collect, transport, and distribute milk in larger quantities. Most of these use milk cans and employ motor transportation, and a limited number of them pasteurize the milk before distribution.

There are many so-called dairies in India. Of these, only a few large dairies in cities are modern in operation. These dairies process, bottle, and distribute milk. The other, smaller dairies possess only a few milk cans, weighing scales, measures, and buckets, and do not pasteurize the milk. Though the number of dairies has increased recently, there has been little or no improvement in their methods of operation.

In India, the pasteurization of milk has not been extensive, because of lack of centralized and limited milk supply and because it involves the use of expensive equipment and skilled personnel. The scattered production of milk makes it difficult and expensive to collect sufficient quantities to operate a pasteurizer efficiently. Pasteurization was first used in India on military farms, by the British Government, to ensure a supply of safe milk for their troops. Today, it is confined to a few large government-civilian research and military farms, educational institutions, a few milk cooperatives and privately owned dairies in big cities. At the present time there are only about one dozen privately owned organizations which pasteurize milk.

Consumption. In spite of the great need of milk in the Indian diet, and in spite of the huge cattle population, the average per capita consumption of milk, including milk products, is only 5.5 oz. per day, varying between 2.6 oz. in the east and 19 oz. in Saurashtra in the west. This is in contrast to the daily per capita consumption in many Western countries of over 2 lb.

As a rule, before consuming milk, it is boiled for hygienic reasons and for keeping purposes. Even pasteurized milk is boiled, because people have acquired a taste for the cooked flavor.

TABLE 1

Total milk production and utilization in India[a]

	Quantity	Total milk produced	Milk converted into products
	(million lb.)	(%)	(%)
Total milk produced	39,631		
Consumed as:			
Fluid milk	14,328	36.3	.....
Ghee	17,161	43.3	67.8
Dahi	3,608	9.1	14.3
Butter	2,485	6.3	9.8
Khoa and Channa	1,642	4.1	6.5
Cream	243	0.6	1.0
Ice cream	164	0.4	0.6

[a] Reported by Basu and De *(2)* and Rangaswamy *(12)*.

Milk products. The palatable life of fresh, unprocessed milk is short in India, owing to the warm climate and unhygienic conditions of production and handling. Most of the milk is converted into products with longer keeping quality and about 36% of the supply is used as fluid milk. The approximate quantities of milk consumed as fluid milk and as various milk products are shown (Table 1). A description of the methods of manufacture of more common Indian milk products, and of the ways in which they are consumed, follows:

Ghee is simply a form of dry butterfat. It is most commonly manufactured by fermenting milk with a starter, and churning butter from 10- to 16-hour-old "curd." The butter is then heated in a pan over an open fire to evaporate moisture. The top scum is removed and the clear butterfat, called "ghee," is strained or decanted off. A substantial amount of fat is lost in the process. A less commonly used method involves separating cream and making creamery butter for the manufacture of ghee.

Well-prepared ghee can be stored for from 9 to 12 months without spoilage. It is commonly used as a shortening and for other cooking purposes. In many locales, ghee is the only form in which milk fat is ingested. About 43% of the milk produced in the country is used for the manufacture of ghee. Because ghee is a fairly expensive product, it is commonly adulterated with various vegetable fats, in order to lower its price. The cheaper vegetable fats are used in place of ghee by a large segment of the population, but ghee is, nevertheless, used even by the poor for special meals and on special occasions *(1)*.

Dahi, curd, or lassi is an Indian counterpart of yoghurt, acidophilus milk, or buttermilk. To make this product, milk is boiled, cooled, and a small quantity of culture, generally the previous day's curd, is added. The milk is then incubated for from 10 to 16 hr. by placing the container near an oven or in some other warm place. The resulting curd is eaten as it is, or beaten with a wooden ladle into a liquid consistency before consumption. In the latter case, it is called "lassi." Lassi is also the name given to the buttermilk obtained in the manufacture of ghee. Dahi is commonly used as a part of a meal and is highly relished. Bread and lassi are a common lunch among the farmers.

Butter. As mentioned under ghee, butter is manufactured either by churning curd or cream. The latter method is followed only in a few dairies in big cities, and at a few butter factories, one of which manufactures about 5,000 lb. of butter daily. Because of the warm climate, only a limited quantity of the butter produced is used as a spread, by wealthy people who are able to keep it chilled. Like ghee, butter is also used for cooking and shortening.

Khoa is a dough-like substance consisting mainly of desiccated milk solids. It is manufactured by boiling milk in a shallow, round-bottom pan *(4)*. During boiling, the milk is stirred constantly in circular motions to avoid burning, and milk sticking on the sides of the pan is continuously scraped loose and allowed to drop back. Finally, the concentrated milk is stirred vigorously to achieve a smooth texture, and drying is continued till the entire mass assumes a dough-like consistency. It is commonly used as a dessert in the form of patties or balls, or in the manufacture of dairy sweets.

Channa is manufactured by heat coagulation and acid precipitation of milk proteins and fat *(4)*. Milk is brought to a boil and acidified either with citrus juice or fresh whey. The coagulated mass is collected by filtration and squeezed to remove excessive whey. The resulting milk coagulum, or channa, is used for the manufacture of various Indian dairy sweets.

Cream. About 0.6% of the total milk produced in the country is consumed as fresh cream, which is separated centrifugally as in the Western countries. It is used in desserts, coffee, and tea, by well-to-do families in cities.

Ice cream. About 0.4% of the total milk production is used for the manufacture of ice cream. There are only a few dairy plants which manufacture ice cream in any appreciable quantity. There are no standards for ice cream, and most of it is manufactured in small, hand-operated freezers. For the most part, the ice cream available is low in fat and milk solids and could be compared to American sherbets. Nevertheless, good-quality ice cream is also available in large cities. Needless to say, it is too expensive for consumption by the average Indian.

Malai may be considered as a by-product obtained from milk as it is being boiled. The skin formed at the air: milk interface during heating is continuously removed and collected. The amount of fat entrapped in the milk skin depends upon the slowness of heating and the time allowed for the fat to rise. Malai is used either as a dessert or as spread.

Indian cheese. Indian cheese is often called Panir *(4)*. There are three distinct varieties of cheese, manufactured in only certain parts of India—Surti, Bandal, and Dacca. All the cheese varieties are soft and unripened, and have to be consumed within a short time due to their poor keeping-quality. Milk is coagulated with rennet and starter, and the set curd is placed on a thin cloth in a straw basket to drain off the whey. Surti cheese is a clot of fresh curd from which the whey has been drained off. Bandal is slightly smoked after the whey has been removed. In the case of Dacca cheese, after the whey is removed by draining, the curd is pressed between heavy weights and then allowed to harden at room temperature for about two weeks. The hardened cheese is then smoked in wood or cowdung smoke. The smoked cheese can be kept well for from four to eight weeks.

None of these cheeses are manufactured in any appreciable quantity, for reasons of religious sentiment which prohibits use of animal products for consumption. Attempts have been made to substitute vegetable rennin in the manufacture of cheese, in order to encourage the use of cheese in India.

Western dairy products. Nearly all Western dairy products are available in the country. They are imported mostly from Holland, Denmark, Switzerland, Australia, and New Zealand. The quantities imported, however, are extremely small.

In 1945-46, India imported only about two million dollars worth of dairy products. Nearly 90% of this amount was spent on condensed and evaporated milk, milk powder, and baby foods, 9% on cheese, and about 0.5% on butter and ghee. Although in recent years the imports have increased considerably, they form only a very small percentage of the total milk and milk products used. In 1953, Khurody *(9)* reported that nearly 14 million dollars worth of products were imported annually.

Evaporated and condensed milk is commonly used in the manufacture of ice cream and other Indian milk products, and as a substitute for coffee cream. A small quantity of whole milk powder is imported. Skimmilk powder forms a large imported item. It is used for reconstitution into a liquid milk, for use where a fresh milk supply is unavailable. A large quantity of skimmilk powder is used by the Governments of Bombay, Madras, and Calcutta in the manufacture of "toned milk." In order to provide fresh milk at a lower price to poor people, the Government of Bombay initiated the manufacture of blended milk, called toned milk (3.5% fat and 9.0% S.N.F.), by mixing rich buffalo milk, skimmilk powder, and water. The Government of Bombay alone uses about 120 tons of nonfat milk powder every month. The consumption of cheese and baby foods is largely dependent upon foreign nationals and some wealthy Indians who have developed a taste for these products.

Possibilities of Improving Dairying in India

At the present time, the basic problem of the country is to increase milk production. This can best be achieved by increasing the milk yield per animal. Better agricultural techniques, improved feeding, breeding and management practices, removal of uneconomical animals, and enlarging and modernizing educational and extension services are some of the methods to be used.

Five-year plan. In order to improve Indian agriculture and industry, five-year plan was instituted in 1951, to make the country self-sufficient in the basic food requirements. The plan envisaged an expenditure of 486 million dollars on agriculture, including approximately 44 million dollars for the improvement of animal husbandry and milk industry *(6)*. While the targets of certain cereals and grains production were achieved in 1956, dairying did not improve significantly.

A second five-year plan has come into being since 1956, under which a sum of from 120 to 125 million dollars has been earmarked for the development of dairy and animal husbandry *(8)*. The program requires the removal of the surplus cattle from the urban areas and improving the performance of the better animals. A large number of improved bulls are to be raised, and breeding is to be controlled through artificial insemination centers operating from key villages. It is thought that better agricultural methods will make available larger quantities of animal feed. About 1,900 additional veterinary dispensaries are to be started. The plan provides for training of personnel for the administration of animal husbandry and dairy programs. The establishment of 36 milk-supply unions, 12 cooperative creameries, and 7 milk-drying plants is contemplated.

Feeding. Datar Singh *(3)* has reported that there is a great need for increasing cattle feed. The present cattle population requires approximately 380 million tons of green fodder, 230 million tons of dry fodder, and 80 million tons of concentrates; whereas, only 169, 135, and 8 million tons of these feeds, respectively, are available. Modern agricultural practices and removal of uneconomical animals would make more feed available for bettering the animals.

Breeding. Mechanization of farming will reduce the need of the bullock, which can be exported for beef. This will eventually reduce the need for dual-purpose breeds (for dairying and draft), and more emphasis can then be given to the improvement of dairy breeds.

A selective and preplanned breeding program would effectively improve the quality of animals. Efforts should be expended to improve the better milk breeds like Red Sindhi, Gir, Sahiwal cows, and Murrah buffaloes.

In the past, efforts have been made to cross-breed local animals with Ayrshire and Holstein bulls. Although the first cross-breds showed increased production the later generations, with a greater percentage of Western blood, could not withstand the rigorous Indian climate. Now work is under way to cross-breed Indian cows with Jersey bulls in the rainy, cooler, and hilly regions *(13)*.

Bulls of unknown and low milk-producing pedigrees should be castrated, and promiscuous mating should be discouraged. It would be desirable to make available breeding facilities for scattered animals owned by farmers who may not have good stud bulls. There is a great deficiency of good-pedigree stud bulls and, for this reason, artificial insemination could be used more effectively. A number of insemination centers have been started by the govern-

ment and future plans include additional breeding facilities and maintenance of pedigree herdbooks.

Management. In order to improve the milk yield, proper feeding and breeding practices and proper management are needed. Owing to unsanitary housing conditions, various animal diseases are prevalent. The farmers should be induced and encouraged, by loans and other incentives, to build proper cow-sheds. Adequate veterinary help at reasonable cost should be made available to farmers through government agencies. Well-equipped mobile veterinary hospitals, manned by qualified personnel for treating sick animals, are necessary. Preventive inoculation of cattle against contagious diseases, already started by the Government, should be extended. In this respect, the Indian Veterinary Research Institute is doing the tremendous job of preparation, distribution, and administration of various vaccines and sera.

Cooperative milk societies, milk unions, and dairy farms. At the present time, a large percentage of milk consumed in urban areas is obtained from surrounding villages. Due to scattered production and difficulties involved in collection and transportation, large quantities of milk are converted into milk products, resulting in a lower income for the milk produced. Therefore, establishment of cooperatives for organized production, collection, processing, transportation, and marketing would result in higher income for farmers.

For some time a few cooperative organizations for the collection and marketing of milk and milk products have been in operation in various parts of India. The farmers participating have been able to sell more milk for fluid consumption. The milk is collected from the farmers at a central point, and then transported in larger quantities. Due to handling larger quantities, the cooperatives have been able to use better means of transportation. In some cases they also process the milk before marketing. A very fine example is set by the Kaira District Cooperative Milk Producers Union at Anand. Sen *(13)* has reported that the Union handles about 10,000 gal. daily, most of which, after pasteurization, is sent to Bombay for repasteurization and distribution by the Aarey Milk Dairy. A similar cooperative organization collects, processes, and distributes about 32,000 lb. milk daily in Madras. Basu and De *(2)* have reported that six such unions have begun work along similar lines, to supply milk to six big cities in the state of Uttar Pardesh.

Removal of cattle from city limits. One of the first projects to remove cattle from the urban areas was started by the Government of Bombay in 1950-51. Under the guidance of Mr. D. N. Khurody, Milk Commissioner of Bombay, the government started a dairy farm about 25 miles from Bombay, called the Aaray Milk Colony, where 15,000 buffaloes are housed, fed, bred, and managed by scientific and modern methods. Here about 17,000 gal. of milk are produced daily, pasteurized and bottled, and sent to Bombay for distribution twice daily.

The Aarey Milk Dairy also receives milk from the Kaira District Cooperative Milk Producers Union for pasteurization and distribution. It functions on a nonprofit basis. It is reported *(13)* that many such projects to remove cattle from the urban areas are in various stages of planning and implementation for Calcutta, Madras, Delhi, and other cities.

Use of surplus milk. Because of the well-pronounced calving period of animals during winter months, there is a greater production of milk during November to January *(9)*. The milk yield of the animals is also higher at this time, owing to the availability of good succulent fodder following the rains. During this flush season, in many heavy milk-producing areas, large quantities of milk are converted into ghee, because of the difficulties and expenses involved in transporting the surplus milk to centers of heavy consumption. The resulting large quantities of skimmilk and butterfat rarely find a ready and profitable market. If the surplus whole milk, skimmilk, or buttermilk could be dried for consumption during the scarce periods, or for transport to the places of demand, much waste could be avoided. Provision has been made by the Government for the establishment of seven milk-powder plants in the Second Five-Year Plan, which began in 1956. Recently, with the help of United Nations' International Children's Emergency Fund and the Colombo Plan, a milk-powder plant has been started in Anand *(13)*.

Consideration should also be given to the establishment of sterilized-milk plants in the surplus milk areas. Since the people have already acquired a taste for cooked flavor in milk and milk products, the sterilized milk would find a ready market in India.

Extension work and training of personnel. The success of any program depends upon the extent of interest shown by the people concerned. The government extension service needs to be enlarged and extended. New and modern methods should be introduced for disseminating information. Since most of the farmers in India are illiterate, printed material is of little value unless explained by proper pictorial illustrations. The government has installed radio receiving-sets in many villages, for the listening pleasure of villagers who cannot afford to own personal sets. This medium could be utilized advantageously in broadcasting better methods of feeding, breeding, and managing cattle, and of handling milk. Educational movies could also be utilized to a greater extent. A greater use of animal shows and rewards for outstanding achievements would increase incentive among producers.

The scope of dairy education carried out at the Indian Dairy Research Institute and at various colleges should be enlarged. Emphasis should be placed on training more extension and dairy workers and on holding periodic clinics and offering short courses for people engaged in dairying.

Foreign aid. In order to implement the various projects outlined in the five-year plan for the improvement of Indian agriculture and industry, the Government of India has received generous loans and grants from several agencies of the United Nations and the United States Government, from the Colombo Plan countries, and other nations, and from the Ford Foundation.

Mention was made earlier of the help received from the United Nations' International Children's Emergency Fund and from the Colombo Plan, in setting up a milk-powder plant in Anand, near Bombay. Under the auspices of the Food and Agricultural Organization, Australia, Canada, U.S.A., Netherlands, and New Zealand, two dairy plants are to be set up in Calcutta and Madras for preparing "toned milk." Various Indo-American loan and gift agreements have been completed, under which India would import large quantities of surplus agricultural commodities, including dairy products, from this country.

India has been the recipient also of various technical experts for teaching the local people improved agricultural, industrial, and educational methods. In this connection, the help received by India from the International Cooperative Administration (ICA) of the United States is of great significance. Under this program, India has been divided into five regions, and teams of five to ten experienced educators in various agricultural fields, from American state educational institutions, have been sent to these regions. Each team has been helping Indian agricultural colleges in the region to enlarge, reorganize, and set up educational, research, and extension programs on the pattern followed by the American land-grant universities. Taking part in the project are The Ohio State University, the Universities of Illinois, Missouri, and Tennessee, and Kansas State College. More recently, the services of a dairy engineer from The Ohio State University were made available to the Government of India, to help plan and design several dairy plants in the country.

Heads of two of these teams, Dean L. L. Rummell of The Ohio State University, and Dean L. B. Howard of the University of Illinois, have expressed the opinion that the progress made during the last two years by the visiting teams has been substantial, and that the help has been accepted enthusiastically by India. After the present contract expires, it is believed that recommendations will be made to extend the program for an additional period of time.

Summary

In India, dairying is a more integral and subsidiary part of agriculture than it is in the Western countries. Like many other phases of Indian agriculture, dairying is underdeveloped and backward. References are made to climatic, geographical, agricultural, and socio-economic conditions of the country, which have contributed to the present situation of the milk industry. India possesses nearly one-third of the world's cattle. Of this, about 67 million are milk cows and buffaloes. Per capita production of milk of these animals is low because of lack of adequate animal feeds, improper management, and promiscuous breeding.

In the country, 40 billion pounds of milk are produced annually and per capita consumption is about 5.5 oz. Because of scattered production, warm climate, and absence of organized collection, processing, and distribution, 36% of the milk produced is consumed in fluid form and the rest is converted into milk products.

An attempt has been made to describe the possibilities of improving dairying in India and to explain how the Government of India has initiated various projects and has received generous help from foreign countries to learn improved agricultural practices. Through improved feeding, management, and breeding practices, it is hoped that within from 10 to 15 years the milk production will be increased considerably, and that efficient collection, processing, and distribution will lead to better prices for the producer and an increased availability of milk for the consumer.

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TECHNICAL NOTE

PROBABLE ROLE OF THE STRECKER DEGRADATION OF AMINO ACIDS IN DEVELOPMENT OF CHEESE FLAVOR[1]

The chemical nature and development of cheese flavor is still rather obscure. Daere (*3*) has likened this situation to that which prevailed in the butter and cultured milk industry before the discovery of the significant organoleptic role of diacetyl in starter cultures. He suggested that an unknown compound may play a prominent role in cured cheese flavor, analogous to that of diacetyl in butter. Others believe that cheese flavor results from blends of large numbers of biochemical products formed during ripening. Harper and Kristoffersen (*4*) recently presented an outline of these products, together with some of the classical formation schemes. The following note presents some observations regarding the development of cheese flavor in model systems, and a theory to stimulate thinking on the problem.

The most comprehensive and informative work on the chemistry of cheese flavor is the 50-year-old report of Suzuki, Hastings, and Hart (*13*) on the relation of volatile fatty acids and esters to the development of Cheddar Cheese flavor. They found that a neutral steam distillate from Cheddar had a pronounced cheese odor, and they inferentially ascribed it to rather simple esters of short-chain acids and alcohols. However, they did not eliminate the possibility of other neutral compounds being responsible for the odor. Some interesting patent claims have been made by Kass (*8*) for the production of baked goods with cheese flavor, by fortifying dough formulas with leucine and/or protein hydrolysates high in leucine content. Analyses of cheese for extractable amino acids, simple peptides, and amines have indicated a role for these compounds in cheese flavor, but the relationship is obscure and some of the reports are contradictory (*11*). Corbin (*2*) found that steam distillates from Cheddar contained small quantities of different neutral carbonyl compounds. Bassett and Harper (*1*) found trace amounts of both acidic and neutral carbonyl compounds in several varieties of cheese.

An attempt to integrate and reconcile the above reports suggested that the Strecker amino acid degradation (*12*) may be a very important reaction in the development of cheese flavor. This reaction is the degradation of an amino acid to an aldehyde of one less carbon than the amino acid.

$$R\cdot CO\cdot CO\cdot R + R'\cdot CHNH_2\cdot COOH = R'\cdot CHO + CO_2 + R\cdot CHNH_2\cdot CO\cdot R$$

The reaction is the basis of the well-known ninhydrin test for amino acids. All of the necessary reactants for the Strecker degradation are found in cheese (amino acids, keto acids, and other dicarbonyl compounds). The reaction is facile; heating solutions of amino acids and dicarbonyl compounds yields rapid reactions, and it is reasonable to expect that the reaction might take place by pure chemical means (without enzyme catalysis) during the cheese-ripening period. Morgan and coworkers (*7, 9*) have demonstrated the presence of enzyme systems in *Streptococcus lactis* var. *maltigenes* which degrade leucine to 3-methylbutanal, and while this biochemical transformation is not strictly a Strecker degradation, the result is the same as far as aldehyde production is concerned. Patton (*10*) has reported that the Strecker degradation of methionine to methional (3-methylthiopropanal) is catalyzed by sunlight and riboflavin and the aldehyde is mainly responsible for sunlight flavor in milk. Hodge (*5*) states the Strecker degradation is an important pathway in the browning reaction in foods. It is apparent that the reaction is probably more significant than has been generally recognized in wide areas of food chemistry.

Preliminary experiments were conducted on a number of milk protein hydrolysates, to obtain clues as to the possible role of the Strecker degradation on the development of cheese flavor. Commercial enzyme preparations (trypsin, pepsin, and/or papain) were added to skimmilk or five per cent casein sols at various pH's from 4.0 to 7.0. After incubation at room temperature for about 18 hours, the hydrolysates were distilled in the presence of added pyruvic acid, isatin, or ninhydrin. In all cases the distillates had strong, suffocating odors. Dilution of the distillates made the odors more pleasant. During these experiments the odors from the distillates permeated the air of the entire building. Proper dilution of the odors in the air resulted in pronounced characteristic cheese odor. Visitors, who had no knowledge of the laboratory work, commented on the cheese odor in the building, and many said it reminded them of toasted cheese. Addition of small amounts of the distillates to cottage cheese curd imparted a definite Cheddar-type flavor to the curd. Synthetic recipes were prepared from a base of equal quantities of cottage cheese curd and 40% cream blended with two per cent added salt. Synthetic chemicals were added to this base and, after thorough mixing in a household cake mixer, the recipes were tasted. The various

[1] Scientific Article No. A624. Contribution No. 2806 of the Maryland Agricultural Experiment Station, Dairy Department.

compounds, reported in the literature to have a relation to cheese flavor, were added in the quantities believed to be present in Cheddar cheese. It was observed that the steam distillate from the Strecker degradation of hydrolyzed casein imparted more Cheddar-like character to the flavor than the fatty acids, amino acids, keto acids, lactic acid, diacetyl, etc. In fact, the mere addition of the Strecker distillate to the curd-cream-salt base yielded Cheddar-like flavor, although it is recognized that lactic and butyric acid are needed to impart the characteristic tartness of Cheddar. A synthetic mixture of amino acids of the same proportions as exist in casein exhibited the same general properties as the hydrolyzed casein preparations when submitted to the Strecker degradation.

Individual amino acids were studied for the character of odor yielded on Strecker degradation. A few mg. of each amino acid were placed in individual test tubes with isatin and two or three ml. water containing enough HCl to dissolve the amino acid. The tubes were heated in a boiling-water bath for 15 minutes and then tested for odor via the nose. The following yielded no odor: glycine, tryptophan, arginine, histidine, lysine, aspartic acid, serine, threonine, and tyrosine. The following amino acids yielded the indicated odors: alanine—malty, valine—apple, leucine—malty, isoleucine—malty-apple, proline—mushroom, phenylalanine—violets, cystine—H_2S, methionine—cheesy-brothy, glutamic acid—bacterial agar, norleucine—flowers, norvaline—Cheddar. These results were incongruous, because norvaline apparently is not a naturally occurring amino acid. A sample of norvaline from another commercial source yielded no cheese odor after Strecker degradation. Further study of the flavor-yielding sample of norvaline showed that it was contaminated with a small amount of methionine. This was revealed by paper chromatography (*6*) and melting point analysis of the 2,4-dinitrophenylhydrazones of the carbonyls yielded by the Strecker distillate. The contaminated norvaline yielded mostly *n*-butanal, with a small amount of methional.

This evidence suggests that the Strecker degradation of amino acids to yield various aldehydes plays a prominent role in cheese-flavor development. Undoubtedly, the reaction is accelerated in the toasting of cheese or in the processing of cheese foods, resulting in an intensified cheese-type flavor. However, its role in the development of the flavor of aged cheese should not be overlooked. Aged Cheddar exhibits certain flavor characteristics similar to those of toasted cheese. In view of the well-known reactivity of the aldehydes produced by Strecker degradation of amino acids, it is logical to suspect that reaction products of the aldehydes may also play a role in flavor development. The Strecker degradation theory should be useful to food manufacturers who are interested in producing cheese-flavored foods. Of the various aldehydes derived from casein, it appears that methional from methionine is the most important flavor compound. High concentrations of methional are obnoxious; however, low concentrations (less than one p.p.m.) have pleasant condiment properties, and in the presence of the other aldehydes from casein, methional imparts a cheese flavor. The theory adequately explains the Kass (*8*) patent claims, and it is suggested that methionine instead of leucine was the important flavor precursor in his experiments. His sample of leucine may have been contaminated with methionine, as was our norvaline. The observations should provide a useful working theory for students of cheese-ripening. The work of MacLeod and Morgan (*9*) suggests a degree of specificity regarding the formation of aldehydes from amino acids by certain organisms. It may be possible to select desirable organisms for cheese cultures, by observing their ability to form aldehydes from certain amino acids.

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ABSTRACTS OF LITERATURE

W. O. Nelson, Abstract Editor

BOOK REVIEWS

398. The Freezing Preservation of Foods. Vol. I—Freezing of Fresh Foods. D. K. TRESSLER AND C. F. EVERS, The Avi Publishing Company, Inc., Westport, Conn. 1214 pp. 1957. Third Ed.

To those well acquainted with the two previous editions, we may indicate that the authors have again used the same approach. Thus, they present sufficient theory and principles to give the necessary background, and then present the practical applications in detail.

The book has been completely revised and augmented. This first volume of the third edition, covering the freezing of uncooked foods, is one-third larger than the second edition. The second volume will be devoted to prepared foods, including ice creams, ices, and sherberts, and will be available shortly.

Guest authors, pioneers in their field, have contributed some of the new material. For example, Talburt and Ramage wrote a chapter on dehydrofreezing, which is of special interest. Another new chapter on the principles of freezing concentrated fruit juice is also a valuable contribution. Old chapters have been rewritten and brought up to date.

The authors have successfully attempted a complete treatment of their subject. Thus, the book opens with a discussion of the food freezing industry, present and future. It then outlines the principles of refrigeration in such a manner that the beginner can readily understand them. The section on requirements for refrigeration and freezing-coil area also is well presented. The subject of suitable horticultural varieties for freezing is discussed later. One chapter is on the physical and chemical changes occurring in foods during freezing, storage, and thawing, while another is on retail display cabinets. There are 31 chapters in all. The one of greatest interest is entitled "The Preparation and Freezing of Milk, Cream, Cheese, and Eggs." It includes an extensive literature review on freezing of whole and concentrated milk.

There are 282 illustrations including maps, charts, graphs, drawings, and pictures. Both freezing and processing equipment and procedures are depicted. There is an extensive and up-to-date bibliography at the end of each chapter, and a complete index for this volume. The appendix is essentially a compilation of analytical methods of general interest to the food freezer.

This book is well-written and should be enthusiastically received. M. P. Steinberg

399. Fundamentals of Chromatography. H. G. CASSIDY, Technique of Organic Chemistry, Vol. X, Arnold Weissberger, Editor. Interscience Publishers, Inc., 250 Fifth Avenue, New York 1, N. Y. 447 pp. $9.75. 1957.

Chromatography ranks as one of the most important techniques of chemistry today, and the literature pertaining to this area increases at an ever-quickening rate. Cassidy has not attempted to make his excellent book a compendium of methods nor of abstracts of literature and references but, more wisely he has chosen to present principles of chromatography in a comprehensive and understanding manner. The principles of a procedure do not change, but methods and techniques of performing a test frequently do; hence, a book on methods can quickly become outmoded, while the principles remain unchanged.

The subject matter presented and the organization of the material can be judged from the titles of the different chapters: 1. Introduction; 2. The Nature of Chromatography; 3. The Molecular Interactions on which Chromatographic Separations Rest; 4. General Theory; 5. Gas-Liquid Partition Chromatography; 6. Column Partition Chromatography; 7. Paper Partition Chromatography and Some Closely Related Methods; 8. Adsorption Chromatography; 9. Ion Exchange; 10. Electron Exchange Polymers (Redox Resins or Polymers); 11. Foam and Emulsion Fractionation; 12. On Recognizing and Evaluating Zones; 13. On the Relation of R or R_F to Molecular Structure; 14. On choosing Mobile and Stationary Phases; 15. On Using Chromatography.

The editor chose a form of presentation which, along with the size and style of type, makes the book easy to read, as well as attractive in design.

Fundamentals of Chromatography is a book that workers in the field will wish to have available for reference and guidance.

S. L. Tuckey

400. Dictionary of Microbiology. M. B. Jacobs, M. J. Gerstein, and W. G. Walter. D. Van Nostrand Company, Inc., Princeton, N. J. $6.75. 1957.

This is a useful volume. The coverage of over 5,000 individual entries naturally is not complete, but it is certainly adequate for the purpose of the book, which is that of a quick reference to the more common nomenclature in the various branches of microbiology. Particularly useful is the inclusion of common prefixal and suffixal terms, and the separate listing of all of the bacterial genera, and many of the genera of yeasts and molds.

The definitions for the most part are not definitive, but a good, casual, understandable meaning is supplied for each term, and for most users this will enhance rather than reduce the book's utility. Anyone who has suffered the problem of finding the meaning of a specialized word or term in the broad field of microbiology will welcome this publication.

L. D. Witter

DAIRY BACTERIOLOGY

401. Bacteriology of milk held at farm bulk cooling tank temperatures. III. Psychrophiles and their growth. E. H. Marth and W. C. Frazier, Dept. of Bacteriol., Univ. of Wis., Madison. J. Milk and Food Technol., **20**, 93. 1957.

Several genera of bacteria were isolated from raw milks stored at farm bulk cooling tank temperatures. When inoculated into raw milk and held at 38° F., all of the cultures except *Flavobacterium* grew at a modest rate during the four-day storage period. *Pseudomonas* cultures showed appreciable growth during the first two days, but grew at a slower rate the last two days. The *Achromobacter* and *Alcaligenes* cultures grew rapidly for the first three days and more slowly the fourth day. *Pseudomonas* cultures grew more rapidly than *Achromobacter, Alcaligenes,* and *Aerobacter.* The *Flavorbacterium* cultures grew very slowly.

H. H. Weiser

402. Changes in the biacetyl content of creamed cottage cheese caused by organisms associated with spoilage. C. S. Wales and L. G. Harmon, Mich. Agr. Expt. Sta., East Lansing. Food Research, **22**, 2: 170. 1957.

Several cottage cheese spoilage organisms reduced the biacetyl and acetylmethylcarbinol content of creamed cottage cheese when inoculated into the product held at 50° and 40° F. The biacetyl content was increased, however, by *Achromobacter butyri.* Variations in biacetyl and acetylmethylcarbinol which ranged in fresh cheese from 0.7 to 10.4 p.p.m. and from 15 to 50 p.p.m., respectively, were not related to pH, nor could they be correlated with organoleptic quality.

F. J. Doan

403. Proteolytic enzymes from *Pseudomonas putrefaciens*. I. Characteristics of an extracellular proteolytic enzyme system. W. C. van der Zant, Texas Agr. Expt. Sta., College Station. Food Research, **22**, 2: 151. 1957.

A proteolytic enzyme system was detected in the medium used for growth of *Pseudomonas putrefaciens* 12. It exhibited maximum activity toward milk proteins at pH 7.0 to 7.5. The system was heat-labile at 55° C. for 10 minutes, or at 60° C. for 3 minutes, and was precipitated by 50 to 75 per cent saturated ammonium sulfate.

F. J. Doan

404. Proteolytic enzymes from *Pseudomonas putrefaciens*. II. Characteristics of an endocellular proteolytic enzyme system. B. J. Camp and W. C. van der Zant. Texas Agr. Expt. Sta., College Station. Food Research, **22**, 2: 158. 1957.

No peptidase activity was found in the extracellular enzyme system of *Pseudomonas putrefaciens* 12. However, extracts of washed cells, disintegrated by sonic vibration, contained several such enzymes, with maximum activity at pH 7.0 to 8.0. They were heat-stable at 60° C. for 8 minutes at pH 7.0, but lost activity under heat treatment on either side of neutrality. Mn and Mg ions were mildly stimulatory to the enzymes, while Cu, Zn, and Ni were inhibitory.

F. J. Doan

405. Effect of the plating medium on the survival of heat-treated cells of *Pseudomonas fluorescens*. C. D. Heater and W. C. van der Zant, Texas Agr. Expt. Sta., College Station. Food Research, **22**, 2: 164. 1957.

Cells of *Pseudomonas fluorescens* which survived heat treatment grew more abundantly on complex media than on simple synthetic media. The addition of glutamic acid, proline, histidine, glycine, and methionine to the synthetic media encouraged growth of such cells. F. J. Doan

406. Some observations on testing milk samples for antibiotics. R. A. Hibbs and J. C. Boyd, Dept. of Dairy Husbandry, Univ. of Idaho, Moscow. J. Milk and Food Technol., 20, 109. 1957.

Growth-inhibiting substances in milk samples were determined by Difco bioassay, using *Bacillus subtilis* as the test organism. The bioassay method was suitable for the detection of penicillin, but not other antibiotics which may be present in milk. The authors believe that additional test organisms should be used. Milk samples may be stored in a frozen condition for 12 weeks without influencing the assay for antibiotics.

H. H. Weiser

407. Kan membranfilter metoden med fordel brukes i den bakteriologiske driftskontroll? (Can the membrane filter method be used advantageously in bacteriological quality control?) S. FUNDER AND S. JOHANNESSEN, Oslo Univ. Nordisk Mejeri-Tidsskrift, **23**, 41. 1957.

The membrane filter method was more time-consuming, more expensive, and gave lower counts than the plate-count method when applied to milk.

Pasteurized milk was diluted 1:10, and raw milk 1:1000. One ml. of each dilution was used for determination of coliforms, and 0.1 ml. for total count. After filtration, the milk was followed by 25 to 30 ml. of sterile water, whereupon 2.5 ml. of the regular media without agar were added to the filter disks.

The counts on the filters averaged 1.87 times lower than the control counts. The savings on preparation and cost of media for the membrane filter method were not sufficient to compensate for the cost of the filters and the five- to six-fold increase in over-all handling time.

T. Kristoffersen

DAIRY CHEMISTRY

408. Ueber das Vorkommen des Spurenelementes Nickel in Nahrungsfetten und anderen Nahrungsmitteln. (The presence of the trace element nickel in edible fats and other foods.) H. LAGONI AND D. MERTEN. Kieler Milchwirtsch. Forschungsberichte, **9**, 1: 73. 1957.

The nickel contents of several samples of butter and vegetable fats ranged from 0.001 to 0.006 p.p.m., with no significant difference between the two types of fat. One sample of hardened technical vegetable fat contained 26 p.p.m. of nickel. Compared to the nickel contents of other foods reported in the literature, the nickel content of edible fats must be considered insignificant. T. Kristoffersen

409. Spurenelemente in Milch, Milcherzeugnissen, und anderen Nahrungsmitteln. (Trace elements in milk, milk products, and other foods.) H. LAGONI, A. WORTMANN, AND D. MERTEN. Kieler Milchwirtsch. Forschungsberichte, **9**, 1: 79. 1957.

A summary of the results reported in the literature regarding trace elements in milk, milk products, and other foods. T. Kristoffersen

ICE CREAM

410. How to avoid costly shutdowns in the ice cream plant. W. H. MARTIN, Kansas State College, Manhattan. Ice Cream Trade J., **55**, 5: 14. 1957.

Causes of costly shutdowns in ice cream plants may result from failure of machines to function properly, improperly trained personnel, and inadequate production planning. A plant should develop a good maintenance program and provide the necessary facilities to keep equipment in good repair. A regular overhaul schedule is a necessity and all employees should be instructed on how to operate the equipment. Each plant should develop a production plan for the entire year to make the best use of the labor, equipment, and facilities available. W. H. Martin

411. How Breyer uses HTST pasteurization. J. J. SHUTT, Breyer Ice Cream Co., Philadelphia, Pa. Ice Cream Trade J., **53**, 5: 46. 1957.

Some of the problems which had to be solved when a HTST pasteurizer was installed were: how to blend cold mix ingredients, how to maintain a constant supply of cold mix at a constant level in the surge tank, and how to avoid air incorporation in the mix ingredients before and during the pasteurization process. The first problem was solved by installing a Norman blender which made it possible to mix cocoa and stabilizer into a cane sugar syrup without heating. The second problem was solved by using a special ball float and two micro-switches which control the mix level, and a pump which delivers slightly more mix than the rated capacity of the pasteurizer. Precaution is taken to avoid air incorporations. A water-sealed centrifugal pump is used to supply the surge tank and the raw mix to the heating side of the regulator plate. Other details of the system and its operation are presented.

W. H. Martin

412. Straight-line flow of products features Borden's newest plant. Anon. Ice Cream Trade J., **53**, 5: 20. 1957.

The Borden Company at Columbus, Ohio, recently put into operation a new two million-gallon capacity ice cream plant. The one-story building, which contains 53,000 sq. ft. of floor space, is designed for the production of excellent quality ice cream efficiently and with the minimum of effort. Some of the distinctive features of the plant include automatic mixing equipment, ice cream hardening tunnel, novelty machine, heating and ventilating systems, and the palletizing of supplies and finished products. W. H. Martin

413. The current status of stabilizers and emulsifiers. B. R. WEINSTEIN, Crest Foods Co., Ashton, Ill. Ice Cream Trade J., **52**, 10:114. 1956.

Stabilizers are classified into two major groups. The carbohydrate-type includes locust bean gum, guar seed gum, Irish moss, and the algins. The protein-type includes gelatin and milk proteins. In selecting stabilizers, factors which should be considered include the ease of incorporation, effect on viscosity, ability of the stabilizer to retard ice-crystal growth, meltdown characteristics of the ice cream, and the effect on flavor and of cost. The three types of emulsifiers used in ice cream include mono and di-glyceride, polyoxythylene stearate, and egg

yolk. The primary function of an emulsifier in an ice cream mix is as a foaming agent. Emulsifiers promote faster whipping and a drier product, reduce tendency for the mix to churn, assist in making good ice cream when refrigeration is inadequate or when poor-whipping freezers are used. They also aid in producing a finer texture and smoother product.
W. H. Martin

414. Dietetic frozen desserts. Anon. Ice Cream Trade J., **52**, 3:14. 1956.

Attention is directed to the size of the dietetic market by giving data from other foods, and by showing how ice cream manufacturers are packaging and pricing dietetic frozen desserts. The low-calorie market can be expected to continue to expand in the future.

Dietetic or diabetic products can be identified as ice cream, ice milk, or frozen desserts. Labeling and identification are controlled by state laws which vary from state to state.
W. H. Martin

415. Refrigeration and ice cream hardening. L. Buehler, Jr., Creamery Package Mfg. Co., Chicago, Ill. Ice Cream Trade J., **52**, 5:32. 1956.

The use of a two-stage booster compressor is recommended for temperatures between −15° to −20° F. An intercooler is used between stages for greater operating efficiency. Hardening of ice cream can be speeded up by spreading out the packages and by air circulators. Although it takes longer to harden ice cream at −20° F. in a hardening tunnel than at −40° to −50° F., more efficient operation is possible without sacrificing quality. The hardening tunnel will speed up hardening; however, a larger refrigeration system will be needed. W. H. Martin

416. The injection method for fruit ice cream. W. A. Krienke, Fla. Agr. Expt. Sta., Gainsville. Ice Cream Trade J., **52**, 1:38. 1956.

A modified method of processing fruit has been developed at the Fla. Agr. Expt. Sta. that requires no heat treatment of the fruit or of the finished injection material. When held in the frozen state or at temperatures around 0° F., the product has an excellent keeping quality. The fruit products processed by this method include frozen concentrated pieces of oranges, limes, lemons, and tangerines. The preparation and use of the fresh-fruit injection material is described. W. H. Martin

417. New developments in ice cream freezing equipment. E. H. Forster, Cherry Burrell Corp., Cedar Rapids, Ia. Ice Cream Trade J., **52**, 12:30. 1956.

Improvements in operation efficiency and automatic controls have been made to meet the new requirements brought about by the increased use of automatic packaging equipment and an increased variety of packages and products. A new freezer has been designed to meet their requirements. It consists of 3 freezing cylinders, each complete with its own drive and control system, giving the equivalent of 3 freezers in one stainless steel case. Each cylinder has a capacity range of 12.5 to 50 gallons of mix per hour, with a refrigerant temperature of −17° F.

To meet the need for greater freezing capacity, a freezer which will handle up to 250 gallons of mix per hour has been built. Other new developments include new air-operated back-pressure regulating valves, removable freezing tubes, and stainless steel displacement-type dashers which minimize the volume of mix in the freezer. W. H. Martin

418. A review of current steps in processing of ice cream. N. Kohl, Southern Dairies, Atlanta, Ga. Ice Cream Trade J., **53**, 3:34. 1957.

The importance of mix-making in the manufacture of high-quality ice cream is discussed. Detailed directions for each step in the process are given and a continuous mix-making setup is described. W. H. Martin

419. Vertical frozen-milk machine. P. Martin (assignor to Marcus Lipsky). U. S. Patent 2,788,643. Seven claims. April 16, 1957. Offic. Gaz. U. S. Pat. Office, **717**, 3:464. 1957.

A device for freezing milk drinks consisting of a vertical cylindrical chamber, containing rotating dasher operated from below, and an inlet for introducing the product to be slushed and a drain or discharge valve for removing the frozen material. R. Whitaker

420. Refrigerated article dispensing apparatus. S. L. Childers (assignor to Vendo Co.). U. S. Patent 2,791,889. Nine claims. May 14, 1957. Offic. Gaz. U. S. Pat. Office, **718**, 2:254. 1957.

A vending machine is described for dispensing individual portions of ice cream or other refrigerated items. R. Whitaker

421. Machine for producing solid molded bodies, especially ice cream bricks. O. G. Hoyer, U. S. Patent 2,791,890. Sixteen claims. May 14, 1957. Offic. Gaz. U. S. Pat. Office, **718**, 2:254. 1957.

A machine for producing molded portions of ice cream and other frozen desserts. The molds travel around an axis and pass through a freezing chamber and a thawing section as they complete one revolution. R. Whitaker

422. Continuous flow, universal dispenser and ice cream filler. W. C. Huntting. U. S. Patent 2,789,588. Nine claims. April 23, 1957. Offic. Gaz. U. S. Pat. Office, **717**, 4:724. 1957.

A piston-type filler is described for filling various shapes and sizes of containers with ice cream as produced in a continuous freezer. The

packages are rotated and vibrated during filling, to insure proper filling of packages.

R. Whitaker

MILK AND CREAM

423. Process for treating fluid dairy products and products produced thereby. A. P. Stewart, Jr., D. Wilcox, and R. A. Johnson (assignor to Foremost Dairies, Inc.). U. S. Patent 2,788,283. Five claims. April 9, 1957. Offic. Gaz. U. S. Pat. Office, **717**, 2:351. 1957.

A sterilized milk having relatively little cooked flavor and stable against the formation of a stale flavor during storage is produced by deaerating raw milk, homogenizing, sterilizing for not over 2 min. at a temperature above 265°, cooling, adding oxygen in an amount equivalent to the reducing substances produced by the sterilization, and sealing in sterile containers under aseptic conditions.

R. Whitaker

424. Daglig bedoemmelse af maelken ved syn, lugt og smag. (Daily organoleptic grading of milk.) A. J. Overby and T. Frandsen. Maelkeritidn., **69**, 33: 1. 1956.

A survey was conducted to determine the daily variations in milk quality, as evaluated organoleptically.

The results showed that the quality of the milk varied greatly from day to day when evaluated by this method. The organoleptic variations were greater than the variations in quality as indicated by the methylene blue test. Payments on the basis of a once-a-week organoleptic examination were not recommended.

T. Kristoffersen

425. Virker en daglig kvalitetsbedoemmelse fremmende paa maelkekvaliteten? (Does a daily quality test improve the milk quality?) A. J. Overby and T. Frandsen. Maelkeritidn., **70**, 9: 1. 1957.

Motivated by the results of the two previously reported surveys, a Danish dairy conducted daily methylene blue tests during the summer of 1956.

Not only did the daily testing result in just quality payments, but the quality of the milk received at the plant improved greatly over that received the previous year.

A statistical analysis of the quality results from this and 14 neighboring plants, using only weekly testing, indicated that the improvement in milk quality was due to the daily sampling, and not to differences in the weather between the summers of 1955 and 1956.

T. Kristoffersen

426. Neuzeitliche Vorsugsmilchgewinnung. (Modern methods of producing high-quality milk.) M. Seelemann and K. H. Wegener. Kieler Milchwirtsch. Forschungsberichte, **9**, 1: 1. 1957. (English Summary).

A guide to producers and supervisory health officers on modern methods of producing high-quality milk.

T. Kristoffersen

427. Forsoeg med daglig kontrol af maelkekvaliteten. (Experiments with daily control of the milk quality.) A. J. Overby and T. Frandsen. Maelkeritidn., **69**, 9: 211. 1956.

Generally, Danish producers are paid for milk according to the quality, as determined by a weekly methylene blue test.

A 5-months' daily survey of the milk from 240 producers demonstrated that quality payment on the basis of a once-a-week test was just. The quality of the milk from 60.6% of the producers was constant. However, the quality of the milk from the remaining 39.4% varied considerably from day to day. The variations in quality could be traced to the irregular cleaning of milking machines.

T. Kristoffersen

NUTRITIVE VALUE OF DAIRY PRODUCTS

428. Nutrition of children in three selected schools in Iowa, Kansas, and Ohio: A pilot study. A. L. Marlott, E. S. Eppright, M. B. Patton, and M. L. Hathaway, Kan. Agr. Expt. Sta. Tech. Bull. 81. 1957.

The study explored procedures and problems relating to the nutrition and dietary habits of school children. The dietary habits were measured by 3- and 7-day food records kept by the children and by the families' weekly food intakes.

Nutritional status was appraised by anthropometric measurements, concentrations of certain blood constituents, and clinical, dental, and skeletal examinations. Attempts also were made to measure socio-economic background and mental maturity and achievement.

A. W. Rudnick, Jr.

SANITATION AND CLEANSING

429. Washing and sanitizing chemicals—automatically. R. B. Hankins, Economics Lab., Inc., St. Paul, Minn. Milk Prod. J., **48**, 4:10. 1957.

The increased use of tankers for hauling milk and milk products has ushered in the need for a means of automatically washing and sanitizing the tankers. The author discusses at length some of the specifications involved in such a system. The system must be fully automatic and follow a predetermined sequence of steps, including pre-rinsing, washing with chemicals, final rinsing, cooling, and sanitizing. In addition, the system must be simple, easy to operate, low in cost, and of rugged construction.

The importance of water analysis is discussed with respect to proper choice of cleaning compounds.

J. J. Janzen

430. Ice cream plant sanitation. N. E. LAZARUS, Lazarus Lab., Inc., Buffalo, N. Y. Ice Cream Trade J., **52**, 4:80. 1956.

Principles of sanitation and sanitizing materials are discussed. Sanitizing agents include hot water, steam, hypochlorites, quaternaries, and iodophors. The latter group has been tested by various laboratories and at Cornell Univ. The iodophors are noncorrosive, nontoxic, and can be used in water of varying degrees of hardness. They solubilize salts associated with the development of milkstone, and there is no perceptible odor, flavor, or taste in use-dilution solutions on butter, cheese and milk, or cream. They are free-rinsing and have sufficient wetting property to insure contact with surfaces to be sanitized, and leave no film deposit. The germicidal action of Iosan and Iobac is effective on contact with practically all types of organisms, including psychrophilic bacteria.

W. H. Martin

431. Milk-waste treatment. L. B. GRIFFITH, U. S. Patent 2,792,202. One claim. May 14, 1957. Offic. Gaz. U. S. Pat. Office, **718**, 2:335. 1957.

A compartmented tank for aerating wastes from a milk plant is described.

R. Whitaker

432. Washing machine for milking equipment. H. H. HOLMSTROM. U. S. Patent 2,791,227. Five claims. May 7, 1957. Offic. Gaz. U. S. Pat. Office, **718**, 1:79. 1957.

An arrangement for cleaning a pipeline milker and parts. A cleaning solution is circulated through the pipe system from a tank. The tank is also used for the hand-washing of teat cups, pails, lids, gaskets, and other accessories.

R. Whitaker

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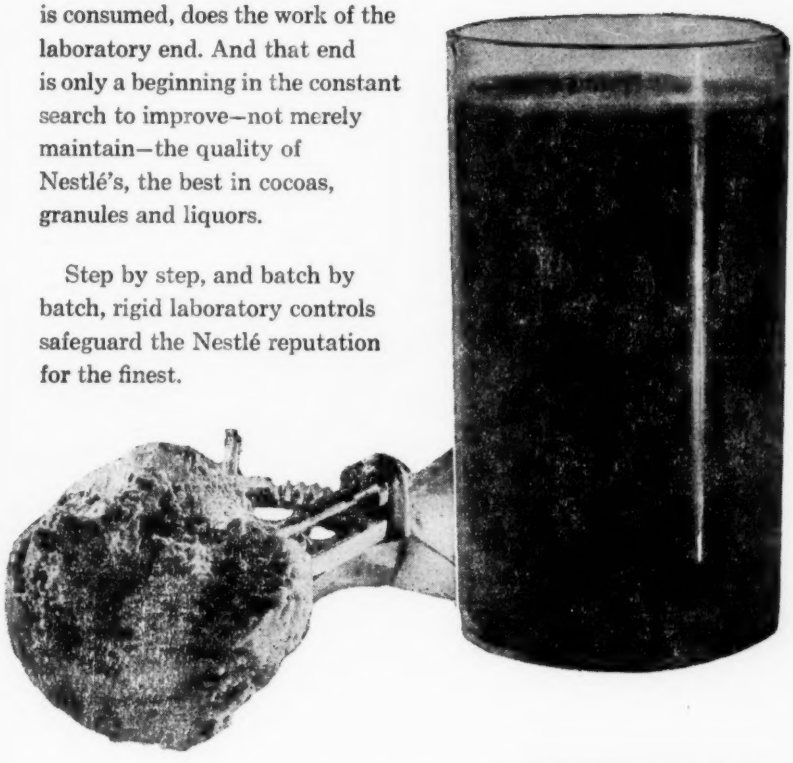

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